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APPLICATION FOR LETTERS PATENT

for

GENES INVOLVED IN IMMUNE-RELATED RESPONSES OBSERVED WITH ASTHMA

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TITLE OF THE INVENTION

GENES INVOLVED IN IMMUNE-RELATED RESPONSES OBSERVED WITH ASTHMA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of pending patent application U.S. Serial No. 10/369,214, filed Feb. 15, 2003, which is a continuation of application No. PCT/NL/01/00610, filed Aug. 16, 2001, designating the United States of America (published in English as PCT International Publication No. WO 02/014366, Feb. 21, 2002), the contents of all of which are incorporated herein by this reference.

TECHNICAL FIELD

[0002] The invention relates to the field of immunology, gene therapy and medicine. Asthma is one of the most common chronic diseases (155 million people worldwide) and is rapidly increasing (20-50% per decade), particularly in children (currently 10% in The Netherlands). Asthma impairs the quality of life and is a major cause of absence from school and work. Asthma, if not treated properly, can be life threatening.

BACKGROUND OF THE INVENTION

[0003] Allergic asthma can be characterized by reversible airway obstruction, elevated levels of IgE, chronic airway inflammation and airway hyperresponsiveness to bronchoconstrictive stimuli, airway tissue remodeling and mucus hypersecretion. The allergic inflammatory infiltrate in the airway tissue predominantly consists of eosinophils and $CD4^+$ T-lymphocytes. It is now widely accepted that type 2 T-helper (Th2) lymphocytes which produce a limited set of cytokines including interleukin-3 (IL3), IL4, IL5, IL9, IL10 and IL13 play an important role in the initiation and progression of allergic asthma (Corrigan and Kay (1992). *Immunology Today. 13*, 501-507; Roinagnani, S. (2000) *J Allergy Clin Immunol 105*, 399-408). Chronic asthma appears to be driven and maintained by persistence of a subset of chronically activated memory T-cells (lymphocytes). Besides T-lymphocytes many other inflammatory cell types are involved in the pathophysiology of allergic asthma such as eosinophils, mast cells,

B-lymphocytes, dendritic cells, macrophages and monocytes as well as resident airway cells such as epithelial cells and smooth muscle cells. Moreover, sensory neurons of which the cell bodies are located in the dorsal root ganglia play an important role in airway inflammation, hyperresponsiveness and cough.

[0004] Currently used pharmacological therapies in allergic asthma only provide temporal symptomatic relief. A more fundamental treatment aimed at antigen-specific T-lymphocytes and antigen-presenting cells is desirable since these cell types play a crucial role in the initiation and progression of allergic asthma. Furthermore, T-lymphocytes may be the only cells that have the potential to induce long-term relief of symptoms. Current therapy for moderate to severe asthma essentially involves multiple classes of molecules: anti-inflammatory glucocorticoids, bronchodilator drugs, and mast-cell inhibitors. The current preferred method is to treat the chronic phase of asthmatic symptoms, as manifested by airway hyperresponsiveness and eosinophilic inflammation, with glucocorticoids to reduce the inflammatory component and hyperresponsiveness (Barnes, 1990; Schleimer, 1990). These drugs are not very selective, targeting non-inflammatory cells as well as inflammatory cells and often have moderate to serious side effects after chronic treatment, especially in children. Furthermore, a subgroup (10%) of asthma patients become relatively resistant to glucocorticoid therapy and increasingly become dependent upon non-glucocorticoid treatment. In addition, there is a strong need for so-called "add-on" therapies to limit the use of high doses of glucocorticoids and the associated side effects. Hence, there is a strong need for a safer, more selective and more efficacious therapeutic which displays a long-term clinical benefit to asthma patients.

SUMMARY OF THE INVENTION

[0005] The invention provides a nucleic acid library comprising genes or functional fragments, derivatives or analogues thereof essentially capable of modulating an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. Modulation herein can refer to up-regulation or down-regulation of an immune response, for example, by activation and/or suppression of gene(s) which are essentially capable of initiation and/or progression and/or suppression and/or repression of an immune response and/or symptoms of the immune response. Modulation herein can also refer, for example, to

positive (i.e., up-regulation) or negative (i.e., down-regulation) regulation of gene transcription, and to the modulation of the gene and gene product. Methods for modulating the expression of genes and gene products are known. The definition "functional fragment thereof" means that a particular subject sequence may vary from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and the subject sequence. An analogue is a compound having functional equivalence or being related to a molecule in question.

[0006] The invention provides a nucleic acid library comprising nucleic acid or functional fragments, derivatives or analogues thereof comprising at least one gene as listed in Table 1, 2 or 3, genes which play an important role in all immune system-related disorders such as all allergic diseases (asthma, rhinitis, atopic dermatitis, urticaria) and auto-immune diseases (i.e., multiple sclerosis). The invention provides a nucleic acid library comprising such genes or fragments thereof, these genes essentially capable of modulating an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma wherein the immune response is up-regulated and/or down-regulated. An immune response herein refers to the physiological response(s) stemming from activation of the immune system by antigens, including immunity to pathogenic organisms and auto-immunity to self-antigens, allergies, inflammatory response and graft rejection. An immune response herein further applies to all immune system-related disorders. Usually the antigenic invader comprises a protein or protein attached moiety. The invention further provides a library comprising genes or functional fragments, derivatives or analogues thereof, the genes essentially capable of initiation and/or progression (i.e., up-regulation) and/or suppression and/or repression (down-regulation) of an immune response wherein the immune responses are airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. The invention provides a nucleic acid or functional fragments thereof selected from those listed in Table 1, 2 or 3, capable of initiation and/or progression and/or suppression and/or repression of an immune response wherein the immune response is asthma. Methods of detecting nucleic acids capable of initiation and/or progression and/or suppression and/or repression of an immune response are known. In one embodiment, such a nucleic acid is derived from a DC-SIGN gene and is described herein. DC-SIGN (signature sequence OtS1-B7) in the primary cultures of bone marrow-derived dendritic cells

demonstrates an important role of this gene and the encoding protein in the cellular function of dendritic cells. Bone marrow-derived dendritic cells or cell lines representing dendritic cells such as the XS52 cell line or other primary cell cultures of this cell type can be used to determine the gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein. Dendritic cells are so-called professional antigen-presenting cells (APC) and thus play a crucial role in the initiation and progression of immune and inflammatory responses mediated by T-lymphocytes. Blockade of mDC-SIGN/OtS1-B7 is beneficial in the treatment of T-lymphocyte-mediated diseases such as allergy, asthma, COPD, auto-immune diseases, inflammatory bowel diseases, allograft rejection and infectious diseases. One of the herein disclosed examples shows that blocking of OtS1-B7 in an asthmatic mouse model results in decreased serum levels of IgE and a decrease in airway hyperreactivity. Blocking of OtS1-B7 is, for example, accomplished by providing a(n) (monoclonal) antibody such as ERTR-9. Allergic diseases are typically accompanied by enhanced levels of (serum) IgE and although IgE can have beneficial effects, for example, against parasitic infections, high levels of IgE are considered to be very detrimental to the health of a subject. Hence, the invention provides a method for at least in part decreasing at least one allergy symptom in a mammal suffering from an allergy, comprising administering to the mammal a substance capable of blocking OtS1-B7 or an equivalent of OtS1-B7. Increased (serum) IgE levels also play a role in asthma and hence the invention provides a method for at least in part decreasing at least one asthma symptom in a mammal having at least one asthma symptom, comprising administering to the mammal a substance capable of blocking OtS1-B7 or an equivalent of OtS1-B7. The term "OtS1-B7 or an equivalent of OtS1-B7" is herein defined as protein (fragment) encoded by a mouse gene with the signature sequence OtS1-B7 or an equivalent thereof in another mammal, for example, a human homolog of the mouse gene with the signature sequence OtS1-B7. Preferably, the blocking substance is a proteinaceous substance and, even more preferably, the proteinaceous substance is a(n) (monoclonal) antibody or a functional equivalent and/or a functional fragment thereof. Polyclonal antibodies and monoclonal antibodies may be produced by methods known to the person skilled in the art. Moreover, a(n) (monoclonal) antibody may further be adapted to the mammal that is in need of treatment. For example, the antibodies may be humanized by known methods. A functional

fragment of an antibody is, for example, the Fab-fragment. A functional equivalent is, for example, a slightly modified antibody by way of deletion or insertions or, for example, by combining the Fab part of an antibody with another Fc-part. An example of an antibody that is used in the method according to the invention is the monoclonal antibody ERTR9 or a functional equivalent and/or functional fragment thereof. An example of a functional equivalent is a (monoclonal) antibody raised against the protein (fragment) encoded by the human homolog of the mouse gene with signature sequence OtS1-B7. Yet other examples of a substance capable of blocking OtS1-B7 or an equivalent of OtS1-B7 are small peptides or small (organic or synthetic) molecules. In yet another embodiment the gene with the signal sequence OtS1-B7 or an equivalent of OtS1-B7 is blocked by anti-sense technology.

[0007] In yet another preferred embodiment, the invention further provides a composition or a pharmaceutical composition comprising a substance capable of blocking OtS1-B7 or an equivalent of OtS1-B7 and a (pharmaceutically acceptable) carrier and/or diluent. More preferably, the substance is a proteinaceous substance, a (small) peptide or a synthetic molecule. Even more preferably, the proteinaceous substance is an antibody or a functional equivalent and/or a functional fragment thereof and yet even more preferably, the antibody is ERTR9 or a functional equivalent and/or a functional fragment thereof.

[0008] Such a (pharmaceutical) composition is very useful in the treatment of a mammal suffering from an allergy and/or an asthma symptom.

[0009] It is clear that the substance may be delivered by a variety of possible routes, for example, via intraperitoneal or intravenous injection, orally or by inhalation. Moreover, the substance may be part of a pharmaceutical composition that further comprises a pharmaceutically suitable and/or acceptable carrier or diluent. A person skilled in the art is aware how to select the proper carrier and/or diluent. The method according to the invention may be used to treat different kinds of asthma symptoms, for example, but not limited to, the treatment of an elevated level of IgE or treatment of airway hyperreactivity. The method according to the invention is furthermore used to decrease the (serum) IgE level in a mammal that suffers from an allergy.

[0010] Furthermore, the method according to the invention is typically applied to a human. Preferably, the invention provides a method for at least in part decreasing asthma symptoms in a mammal suffering from the symptoms, comprising providing the mammal with a

substance capable of blocking OtS1-B7 or an equivalent of OtS1-B7, wherein the symptoms are (completely) decreased. Even more preferably, the (partial) decrease in asthma symptoms results in an improved sense of overall well-being. Nonlimiting examples of asthma symptoms that can be treated according to a method of the invention include reversible airway obstruction, elevated levels of IgE, chronic airway inflammation and airway hyperresponsiveness to bronchoconstrictive stimuli, airway tissue remodeling and mucus hypersecretion. The method according to the invention is furthermore used to decrease the (serum) IgE levels in patients suffering from allergy. Nonlimiting examples of allergies are allergy to dust, allergy to grasses or allergy to certain food ingredients.

[0011] Furthermore, the invention also provides use of an OtS1-B7 blocking substance or the use of an OtS1-B7 blocking substance for the preparation of a medicament for the treatment of allergy and/or asthma.

[0012] In another embodiment, such a gene is derived from a calcium-activated chloride channel gene as also described below. Calcium-activated chloride channels (CLCA1-4) can be blocked by mono- and polyclonal antibodies or fragments thereof directed against the ion channel (protein or peptide fragments); known nonspecific chloride channel antagonists such as 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS), 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPD), niflumic acid, and the anti-allergic drug cromolyn.

[0013] Changes in gene expression underlie most, if not all, pathophysiological processes. A variety of methods for detecting changes in gene expression in a healthy versus a diseased animal, and for detecting a nucleic acid for the formation of a library for the subject of the invention are known. These procedures include, but are not limited to DNA-DNA or DNA-RNA hybridization. The form of such quantitative methods may include Southern or Northern analysis, dot/slot blot or other membrane-based technologies, PCR technologies such as DNA Chip, Taqman®, NASBA, SDA, TMA, *in situ* hybridization, protein bioassay or immunoassay techniques ELISA, IFA, proteomic and metabolomic technologies. These technologies are often found at the basis of commercially available diagnostic kits often used for screening purposes.

[0014] The invention provides a nucleic acid library comprising genes or fragments thereof, the genes essentially capable of modulating an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma wherein the genes comprise a nucleic acid essentially equivalent to a signature sequence as shown in Table 1, 2 or 3. A "signature sequence" herein refers to a marker sequence and/or sequence or any other mode of identification of a sequence (i.e., name). "Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represents the sense or antisense strand. The definition "antisense" RNA is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA: complementary in the sense that each base (or majority of bases) in the antisense strand (read in the 5′ to 3′ sense) is capable of pairing with the corresponding base (G with C, A with U), in the mRNA sequence read in the 5′ to 3′ sense. The definition "sense" RNA is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence. Preferably, the nucleic acid is an immune response gene. An "immune response gene" is any gene that determines the ability of lymphocytes to mount an immune response to specific antigens. The definition "essentially equivalent" means that the subject signature sequence can vary from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which will not result in a functional dissimilarity between the two sequences. It may be advantageous to produce nucleotide sequences, the subject of the invention or derivatives thereof possessing a substantially different codon usage. It is known by those skilled in the art that as a result of degeneracy of the genetic code, a multitude of gene sequences, some bearing minimal homology to the nucleotide sequences of any known and any naturally occurring genes may be produced. The invention includes each and every possible variation of the nucleotide sequences that could be made by selecting combinations based on possible codon choices.

[0015] The invention provides a library wherein the genes encode a regulatory molecule and/or co-stimulatory molecule and/or adhesion molecule and/or receptor molecule involved in modulating an immune response. The definition "regulatory molecule" is an entity which assists the cell in sensing its environment. For example, "a regulatory molecule" can effect an immune response by modulating either positively or negatively gene transcription. The

definition "stimulatory molecule" is an entity which can activate an immune response. The definition "adhesion molecules" is any pair of complementary molecules that bind specifically to one another to effect a positive or negative immune response. The molecule can be any entity which can bind to, for example, nucleic acid, proteinaceous substance or receptor, etc., to effect a positive or negative immune response. The definition "receptor" is an entity to which a ligand binds which triggers an immune response. The definition "receptor molecule" could be, for example, a ligand (i.e., any macromolecule) which binds to a receptor to effect an immune response. A ligand is a molecule that binds to a complementary site on a given structure. For example, oxygen is a ligand for hemoglobin and a substrate of an enzyme molecule is a specific ligand of that molecule. The invention further provides a method for modulating an immune response of an individual comprising modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3.

[0016] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, Gob-5 (signature sequence R1-SO-R1-C11). Gob 5 is a cell membrane protein belonging to the family of calcium-activated chloride channels and discovered in intestinal goblet cells in mice. Human CaCC1 and the identical CLCA1 are most likely the human homologs of murine gob-5. Gob-5 can have another function as a cell adhesion molecule. Northern blot analysis revealed that gob-5 is abundantly expressed in the stomach, small intestine, uterus and slightly expressed in the trachea of mice. *In situ* hybridization demonstrated that gob-5 expression is located in the mucus-secreting cells of these three tissues. In humans, CaCC1/CLCA1 are also primarily expressed in the digestive tract. Gob-5 is expressed in lymph-nodes, lung tissue, bronchoalveolar lavage cells and bone marrow from mice and is up-regulated in these tissues in the mouse asthma model. Mucus secreting goblet cells have never been described in lymph nodes or bone marrow. In addition, Gob 5 is expressed in murine bone marrow-derived mast cells and murine mast-cell lines. Gob-5 plays a role in secretory processes based on its function as a chloride channel. Chloride channels have been shown to be involved in mast cell activation and degranulation since inhibition of

these channels by nonselective broad spectrum chloride channel inhibitors inhibit IgE-mediated rat mast-cell degranulation *in vitro*. Additionally a strong up-regulation of gob-5 in the dorsal root ganglia (DRG) in the mouse asthma model was observed. The expression of other members of the calcium-activated chloride channel gene family by PCR (Table 2) was investigated. Murine homolog of human CaCC3 (EST AA726662) was identified and their expression was shown to be strongly up-regulated in DRG of the mouse asthma model.

[0017] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, LR8 (R1-OS-B1-D3). LR8 belongs to the family of the tetraspanin (4TM) superfamily. LR8 mRNA was not detectable by PCR in human smooth muscle cells, endothelial cells or epithelial cells. Murine LR8 mRNA expression in lymph nodes from mice was confirmed along with a down-regulation in a mouse asthma model. Bio-informatics analysis of the LR8 protein confirmed the presumed 4TM structure of the protein and revealed a striking homology with the beta chain of the high affinity IgE receptor (FcεRI). The tetraspanin superfamily has grown to nearly 20 known genes since its discovery in 1990. All encode cell surface proteins that span the membrane four times, forming two extracellular loops. Many of these proteins have a flair for promiscuous associations with other molecules, including lineage-specific proteins, integrins, and other tetraspanins. In terms of function, they are involved in diverse processes such as cell activation and proliferation, adhesion and motility, differentiation, and cancer. These functions relate to their ability to act as "molecular facilitators," grouping specific cell surface proteins and thus increasing the formation and stability of functional signaling complexes. LR8 is similar to CLAST1, a murine gene that is activated upon ligation of CD40 (Genbank: BAA83596). CD40 is predominantly expressed on so-called "antigen-presenting cells" and ligation of CD40 induces the expression of several molecules involved in the activation and regulation of T-lymphocytes (CD80; CD86; IL12). CD40 is an important maturation signal for dendritic cells. Immature dendritic cells take up antigen in peripheral tissues and migrate to secondary lymphoid tissues (draining lymph node) where they maturate and present antigen to lymphocytes. Several proteins are induced or

down-regulated upon dendritic cell maturation. Many of the differentially activated genes appear to be involved in the modulation (regulation/activation) of T-lymphocytes (Table 1, 2 or 3).

[0018] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, β-Amyloid-precursor like protein 2 (APLP2) (SvO2-1-B7). APLP2 is a highly conserved protein and is located on mouse chromosome 9. Moreover, in an experimental asthma model, airway hyperresponsiveness has been linked to a locus on chromosome 9, syntenic with human 11q24. APLP2 is a member of the Alzheimer precursor protein family including the Alzheimer peptide precursor (APP). These proteins all share three domains of similarity, interdispersed with completely divergent regions. APLP2 is a type-I integral membrane protein that contains a single membrane spanning domain with a large extracellular N-terminal domain and a short C-terminal cytoplasmic domain. APPL2 is ubiquitously expressed. Alternative splicing of APPL2 pre-mRNA generates at least four transcripts. Several functional domains have been identified in APLP2, including a DNA binding motif, an N-terminal cysteine rich domain exhibiting zinc, copper, and heparin binding activity, followed by a very acidic region and, depending on the isoform, the Kunitz protease inhibitor (KPI) domain. Interestingly, the KPI domain inhibits serine proteases like trypsin, plasmin, tryptase and chymase of which the latter two are released by activated mast cells. Tryptase has been implicated in the development of airway hyperresponsiveness. Mast cell mediator serotonin stimulates the release of APLP2 ectodomain (containing the KPI domain). Other functions that have been described for APLP2 are (i) an interaction with MHC class I, (ii) a role as adhesion molecule through interactions with extracellular matrix components, (iii) a role in epithelial wound healing and (iv) a potential role in the inhibition of platelet activation by the N-terminal cysteine-rich domain.

[0019] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. For example, the invention provides a method for the treatment of an immune response more particularly asthma and COPD comprising

providing APLP2 or its KPI domain or by induction of APLP2 expression. APLP2 through the inhibition of the detrimental effects of mast cell proteases, by repair of epithelial damage and by inhibition of platelet activation is capable of treating an immune-related response. Furthermore, many allergens have been shown to have protease activities that appear to be crucial for allergic sensitization. By its KPI domain, APLP2 can inhibit the proteolytic activities of allergens and thereby prevent the initiation and progression of allergic responses. Another effect of the KPI domain of APLP2 is inhibition of the activation of protease-activated receptors (PARs) by serine proteases. PAR2 is involved in bronchorelaxation and protection against bronchoconstriction by stimulating the generation of prostaglandin E2 by airway epithelial cells. However, it was demonstrated that trypsin and a PAR2 ligand-induced bronchoconstriction in guinea pigs *in vivo*, despite the induction of relaxation by these mediators in isolated trachea and bronchi. The bronchoconstriction appeared to be mediated by a neural mechanism since the bronchoconstriction was inhibited by the combination of NK1 and NK2 receptor antagonists. These data suggest that the PAR2 ligand activates sensory nerves. In agreement herewith, trypsin and mast cell tryptase induced a wide-spread neurogenic inflammation initiated by activation of neuronal PAR2 receptors. Inhibition of tryptase and other serine proteases by APLP2 or its KPI domain can antagonize neurogenic inflammation and bronchoconstriction. Moreover, other PARs appear to be involved in inflammation. Activation of these receptors (PAR2) by serine proteases is sensitive to inhibition by APLP2 or its KPI domain. Analogous to intra-membrane cleavage of APP and Notch by aspartyl proteases (γ-secretase, presenilins), APLP2 can be cleaved by these aspartyl proteases since it is homologous to APP in the region (IATVIVI) (SEQ ID NO: 1) where γ-secretase cleaves APP. This cleavage will lead to the generation of the extracellular part of APLP2 and an intracellular part of 57 amino acids, which may directly or indirectly modify the transcription of target genes. The APLP2 C57 peptide contains the "NPTY" sequence (SEQ ID NO: 2), which is present in many growth factor receptors and appears to be involved in cellular signaling. Interestingly, T-lymphocytes have been shown to express presenilin-1 and 2 at the cell surface. Cleavage of APLP2 is involved in T-lymphocyte activation. Another, at present unidentified, protease may cleave APLP2 in its transmembrane region and generate the release of an intracellular peptide containing the "NPTY" sequence (SEQ ID NO: 2).

[0020] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. For example, phosphotyrosine binding (PTB) domains have been identified in a large number of proteins. PTB domains play an important role in signal transduction by growth factor receptors. Several PTB proteins have been shown to bind to amyloid proteins through the "NPTY" motif (SEQ ID NO: 2) like Fe65, Fe65-like, X11 and X11-like proteins, Shc and IRS-1. The interactions of APLP2 with Shc and IRS-1 is dependent on tyrosine phosphorylation whereas the interactions with Fe65 and X11 are not. The Fe65 adaptor protein interacts with the transcription factor CP2/LSF/LBP1. The "NPTY" motif (SEQ ID NO: 2), has been shown to be involved in binding to Shc, a Src homology 2 (SH2)-containing proto oncogene product implicated in activating Ras via association with Grb2 protein. Activation of the Ras pathway involves the MAPK signal transduction pathway which has been shown to be involved in the induction of many inflammatory genes. The Shc/Grb2/Sos complex is also involved in the activation of the Ras pathway in T-lymphocytes. It is unknown whether APLP2 or other proteins of this family with an "NPTY" domain (SEQ ID NO: 2) are involved in T-cell activation and differentiation. Caspases can also cleave APP at the caspase consensus site "VEVD" (SEQ ID NO: 3), leading to the generation of a C-terminal 31 amino acid peptide which contains the internalization sequence "NPTY" (SEQ ID NO: 2). Since APLP2 contains both the caspase consensus site "VEVD" (SEQ ID NO: 3) as well as the internalization sequence "NPTY" (SEQ ID NO: 2), it is clear that APLP2 can also be cleaved by caspases leading to the generation of a C-terminal 31 amino acid peptide which is homologous to the peptide generated by APP cleavage. The APP C31 peptide has been demonstrated to initiate cell death. Apoptosis or cell death is an important mechanism to limit immune and inflammatory reactions. On the other hand, cell death may be unwanted, i.e., death of airway epithelial cells may increase airway responsiveness. The invention provides a method for the treatment and/or prevention of an immune-related response more particularly allergic asthma and related inflammatory diseases and COPD comprising modulating APLP2 or its KPI domain and/or by induction of APLP2 expression. Treatment by providing APLP2 or its KPI domain or induction of APLP2 expression is effective in the treatment of (1) the neurogenic component of inflammatory responses, (2)

hyperalgesia during inflammatory responses, (3) cough due to airway inflammation and (4) bronchoconstriction induced by activation of sensory nerves. Cleavage of APLP2 by presenilins (γ-secretase) or other proteases or by caspase is involved in activation-induced cell death in T-lymphocytes and is involved in the induction of peripheral tolerance.

[0021] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma; for example, the invention provides a method for the treatment of immune responses comprising stimulating the cleavage of the intracellular domain of APLP2 by allosteric activation of proteases or by binding of APLP2 to its ligand together with an antigen-specific stimulation which will induce peripheral tolerance to the antigen. This treatment is effective for allergic asthma and other diseases mediated by T-lymphocytes such as auto-immunity and graft-rejection.

[0022] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, mouse GDP-dissociation inhibitor (Ly-GDI: signature sequence Sv-O2-1-D8). Ly-GDI was originally identified in lymphocytes and likewise called "lymphoid-specific GDI" (Ly-GDI). Independently, Ly-GDI gene was cloned from human and from mouse and the term GDP-dissociation inhibitor D4 was designated. Mouse and human D4-GDI (Ly-GDI) share 89% amino acid sequence identity. Murine Ly-GDI is located on chromosome 6, the human homolog (Ly-GDI or D4-GDI) is located on chromosome 12p12.3. Northern blot analysis demonstrated that Ly-GDI was expressed abundantly in lung, and at lower levels in several other tissues. Another study using Northern blot analysis revealed that Ly-GDI is expressed as a 1.4-kb transcript only in hematopoietic tissues. Antibodies against Ly-GDI recognized a 27-kD protein on Western blots

of B- and T-cell line lysates. It is now generally accepted that Ly-GDI is preferentially expressed in hematopoietic cells and can function as a GDP-dissociation inhibitor of Rho GTP binding proteins (Rac and Cdc42) but with less potency than the ubiquitously expressed RhoGDI. There are three subfamilies of small GTP-binding proteins, Ras, Rho and Rab. The present thinking is that Ras proteins are principally involved in signal transduction and cell proliferation, Rho proteins (Rac1, Rac2, TC10 and Cdc42) regulate cytoskeletal organization and Rab proteins are involved in the control of intracellular membrane traffic. The GTP-binding proteins are active only in the GTP-bound state. At least two classes of proteins tightly regulate cycling between the GTP-bound (active) and GDP-bound (inactive) states: GTPase-activating proteins (GAPs) and GDP/GTP exchange factors (GEF). GAPs deactivate small GTP-binding proteins by stimulating their low intrinsic GTPase activity to cause hydrolysis of GTP to GDP. GEFs are of two types including GDP dissociation stimulators (GDS, alternatively called guanine nucleotide releasing factors (GRF) and GDP-dissociation inhibitors (GDIs). The GDIs decrease the rate of GDP dissociation from Ras-like GTPases. It was found that Ly-GDI bound RhoA, and *in vitro* inhibited GDP dissociation from RhoA. Stimulation of T-lymphocytes with phorbol ester led to phosphorylation (activation) of Ly-GDI. It has been suggested that Ly-GDI may be involved in the regulation of hematopoietic-specific Rho-family GTPases because it is less potent than the ubiquitously expressed Rho-GDI. In T-lymphocytes, Rac and Cdc42 are important Rho-family GTPases involved in T-cell activation. Both Rac and Cdc42 are activated by Vav that has GDS activity (see FIG. 1). Rac and Cdc42 are involved in downstream signaling to the nucleus via the JNK pathway leading to the transcription factors AP1 (fos/jun) and NFAT (nuclear factor of activated T-cells). These transcription factors are involved in transcription of cytokines such as IL1, IL4, GM-CSF, etc. Recently, it was demonstrated that Ly-GDI also interacts with the proto-oncogene Vav. Vav functions as a specific GDS for Rho, Rac and Cdc42 and is regulated by tyrosine phosphorylation in hematopoietic cells. Vav integrates signals from lymphocyte antigen receptors and co-stimulatory molecules to control development, differentiation and cell cycle. Interestingly, Vav knock-out mice have a defective IgE antibody production that can be attributed to compromised T-cell help due to impaired IL-4 transcription. Ly-GDI knock-out mice have been generated and did not show striking abnormalities of lymphoid development or thymocyte selection. The mice also exhibited normal immune responses including lymphocyte

proliferation, IL-2 production, cytotoxic T-lymphocyte activity, antibody production, antigen processing and presentation, immune cell aggregation and migration, and protection against an intracellular protozoan. However, Ly-GDI-deficient mice exhibited deregulated T- and B-cell interactions after *in vitro* cultivation of mixed lymphocyte populations in concanavalin A (Con A) leading to overexpansion of B-lymphocytes. Further studies revealed that Ly-GDI deficiency decreased IL-2 withdrawal-induced apoptosis of lymph node cells while dexamethasone- and T-cell receptor-induced apoptosis remained intact. These data implicate the regulation of the Rho GTPase by Ly-GDI in lymphocyte survival and responsiveness, but suggest that these functions may be partially complemented by other Rho regulatory proteins when the Ly-GDI protein is deficient. Increased expression of GDP-dissociation inhibitor in the mouse asthma model in the lung-draining lymph nodes of "asthmatic" (OVA-challenged) compared to "healthy" (saline-challenged) mice was observed. A role for the GDP-dissociation inhibitor in the generation of Th2 immune responses is provided.

[0023] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance; for example, a mouse fragment (signature sequence R1-SO-R1-A12) homologous to several mouse ESTs and human (Cdc42-GAP) was identified. Human Cdc42 GTPase-activating (Cdc42-GAP) functions as a GAP for the Rho-family GTPase Cdc42 (see FIG. 1). Cdc42 can regulate the actin cytoskeleton through activation of Wiskott-Aldrich syndrome protein (WASP). Mutations in WASP lead to the Wiskott-Aldrich syndrome, a pediatric disorder characterized by actin cytoskeletal defects in hematopoietic cells, leading clinically to thrombocytopenia, eczema and immunodeficiency. Recently, WASP-interacting protein (WIP) was shown to enhance the Vav-mediated activation of NF-AT/AP-1 gene transcription. Moreover, the interaction of WIP with WASP is necessary, but not sufficient for the ability of WIP to regulate NF-AT/AP-1 activity. Both Ly-GDI and Cdc42-GAP function in concert as inactivators of Cdc42. The invention provides a method for the treatment of immune responses more in particular allergic asthma and related allergic and Th2-mediated inflammatory diseases comprising providing blockade of Ly-GDI and/or Cdc42-GAP by selective antagonist(s) which inhibit T-helper lymphocyte type-2 (Th2)

responses. The invention provides a method for the treatment of immune responses, more in particular, Th1-lymphocyte-mediated diseases like auto-immune diseases comprising modulating Ly-GDI and/or Cdc42-GAP, more preferably, inducing the expression of these proteins. Induction of the expression of these proteins induces T-helper lymphocyte type-2 responses and is, therefore, effective in the treatment of Th1-lymphocyte-mediated diseases like auto-immune diseases.

[0024] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, TIS11d/tristetraprolin homolog (signature sequence OtS2-A7). The human TIS11d protein is part of the TIS11 family of proteins also called tristetraprolin protein. These are basic proline-rich proteins and contain an unusual CCCH type of zinc finger structure. Tumor necrosis factor-α (TNF-α) is a major mediator of both acute and chronic inflammatory responses in many diseases. In addition to its well-known role in acute septic shock, it has been implicated in the pathogenesis of chronic processes such as autoimmunity, graft-versus-host disease, rheumatoid arthritis, Crohn's disease, and the cachexia accompanying cancer and AIDS. TIS11 interferes with TNF-α production by destabilizing its mRNA. This pathway represents a potential target for anti-TNF-α therapies. TIS11 deficiency also results in increased cellular production of granulocyte-macrophage colony-stimulating factor and increased stability of its mRNA, apparently secondary to decreased deadenylation. TIS11 is a physiologic regulator of GM-CSF mRNA deadenylation and stability. The invention provides a method for the treatment of an immune-related response, comprising modulating expression, more preferably, increased expression of TIS11d protein which inhibits the development of allergic asthma and related allergic and inflammatory diseases.

[0025] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of

an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, many of the differentially activated genes as listed in Table 1, 2 or 3 are involved in the regulation/activation of T-lymphocytes (T-lymphocyte activation molecules). Those up-regulated genes/proteins included terminal deoxynucleotidyl transferase (signature sequence: R1-SO-R1-E7), CsA-19 (signature sequence: ST-O1-B3), Pendulin (signature sequence: R1-SO-R1-E11), RA70 (signature sequence: STO1-D3), Ly-GDI (signature sequence SVO2-1-D8), Plastin-2 EST (signature sequence: SV02-1-C4), RNA Polymerase-II subunit EST (signature sequence: SV02-1-G3), Clathrin EST (signature sequence: SV02-1-D4), Cdc42-GAP (signature sequence: R1-SO-R1-A12). Those down-regulated genes/proteins were Stat-1 (signature sequence: R1-OS-B1-G3) IL2-R-gamma (signature sequence: OTS2-D9) IFN-γ-R (signature sequence: OTS2-A10).

[0026] The invention provides a method for modulating an immune response of an individual comprising modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3, wherein the gene modulates a signal transduction cascade pertaining to an immune response. Methods for modulating the expression of a nucleic acid are well known. In a preferred embodiment are nucleic acids as shown in Table 1, 2 or 3 and functional equivalents whose products are capable of modulating genes of pathways central to immune response. "Modulating" herein can also mean activation or suppression. More preferable is that the nucleic acid is involved in signal transduction cascades leading to suppression or activation of immune responses. More preferable is that the nucleic acid encodes a proteinaceous substance (e.g., a transcription factor) which may be involved in the activation or suppression of the Ras pathway in T-lymphocytes. Activation of the RAS pathway involves the MAP kinase (MAPK) signal transduction pathway which is involved in the induction of many immune-related genes.

[0027] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, LR8. LR8 is part of a multi-chain Fc receptor and is involved in the signal transduction by this Fc receptor upon ligand (immunoglobulin) binding. The invention provides a method for the treatment of an immune

response comprising providing a blockade of LR8. A blockade of LR8 prevents the activation of inflammatory cells through this Fc receptor. The invention provides a method for the treatment and/or prevention of an immune-related response comprising modulating inhibition of aspartyl proteases such as presenilins (γ-secretase) involved in the cleavage of the intracellular 57 amino-acid part of APLP2 and blockade of the "NPTY" (SEQ ID NO: 2) motif, which prevents activation of downstream signal transduction pathways including the Ras and MAPK pathway and associated changes in gene expression.

[0028] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, Heat-shock protein 84 (Hsp84) (signature sequence: OTS2-C6). Hsp84 is a member of the Hsp90 family of heat-shock proteins. Hsp90 proteins are ubiquitous molecular chaperones with key roles in the folding, activation and assembly of a range of client proteins typically involved in signal transduction, cell cycle control or transcriptional regulation. Hsp90 has been shown to possess an inherent ATPase activity that is essential for the activation of authentic client proteins. Recently, Hsp90 and hsc70 (signature sequence: OTS2-H2) are both necessary and sufficient to activate hormone binding by the glucocorticoid receptor. A deficiency of Hsp90 or Hsp70 proteins may thus decrease the sensitivity of cells to the effects of glucocorticoids. In asthma, a gradual decrease in glucocorticoid sensitivity occurs. This decrease in glucocorticoid sensitivity can be mimicked by several cytokines, e.g., IL-4. The invention provides a method for the treatment and/or prevention of an immune-related response comprising modulating expression, more preferably, increased expression of Hsp90 and/or Hsp70 proteins. This increases the sensitivity to the anti-inflammatory effects of glucocorticoids and is valuable in the treatment of asthma and other chronic inflammatory diseases.

[0029] Transcription factors are directed to the nucleus by their nuclear localization sequence (NLS) in a multistep process. The first step is to dock the NLS-containing protein to the nuclear pore and this is carried out by pendulin and Srp1. Pendulin (signature sequence R1-SO-R1-E11) contains an armadillo repeat region that is involved in NLS binding. Pendulin has been shown to be involved in the nuclear localization of lymphoid enhancer factor 1 (LEF-1)

but not of the highly related T-cell factor 1 (TCF-1). Pendulin is the mouse homolog of human Rch1/Srp1α/importin-α. In contrast to a low-level of expression of mSrp1 and pendulin in all tissues examined, mouse pendulin is highly expressed in spleen, thymus and heart. Pendulin may perform additional or unique functions in tissues that express high levels of this protein. Increased expression of pendulin in lymph nodes of the mouse asthma model was observed. The invention provides a method for treatment and/or prevention of an immune-related response, more preferably, asthma and related auto-immune and inflammatory diseases, comprising modulating expression of pendulin, more preferably, increasing expression of pendulin.

[0030] The invention provides a method for modulating an immune response comprising modulating a gene(s) involved in signal transduction cascades leading to the production of cytokines and/or chemokines and/or growth factors pertaining to an immune response. Cytokines are primarily involved in signaling between cells of the immune system (e.g., IL-4, IL-6, IL-8, IL-17 and Il-18). "Chemokines" are defined primarily as those compounds that draw cells and other factors to sites of injury in the body (e.g., human GRO-β, Human IP-10). Growth factors promote cell division and proliferation of certain cell types (e.g., human transforming growth factor β-1, etc.).

[0031] The invention provides a method for modulating an immune response comprising modulating a gene, wherein the gene is involved in sensory nerve activation involved in an immune response. More preferably, the immune response is an inflammatory response. Chloride channels appear to be involved in neuronal excitability. Dorsal root ganglia contain sensory nerve bodies that are involved in neurogenic inflammation which contributes to allergic inflammation and pain (inflammatory hyperalgesia). Interference with these chloride channels blockade of hCaCC1 (or gob-5) and/or hCaCC3 (or the murine homolog) by selective antagonists can limit neurogenic inflammation in asthma and other diseases with a neurogenic inflammatory component. Furthermore, cough, which is a prominent symptom of asthma, is believed to be a result of sensory nerve activation. The invention provides a method for the treatment of immune-related responses comprising providing blockade of hCaCC1 (or gob-5) and/or hCaCC3 (or the murine homolog) by selective antagonists.

[0032] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid

identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, a blockade of hCaCC1 (or gob-5) by a selective antagonist inhibits mast-cell activation and can be used in diseases in which mast cells play an important role such as all allergic diseases (rhinitis, atopic dermatitis, asthma, urticaria) and auto-immune diseases (i.e., multiple sclerosis). Blockade of hCaCC1 (or gob-5) and/or hCaCC3 inhibits the excitability of sensory neurons and thereby prevents or decreases (1) the neurogenic component of inflammatory responses, (2) hyperalgesia during inflammatory responses and (3) cough due to airway inflammation. Activation of receptors (PAR2) by serine proteases is sensitive to inhibition by APLP2 or its KPI domain and treatment with APLP2 or its KPI domain or induction of APLP2 expression is effective in the treatment of bronchoconstriction induced by activation of sensory nerves.

[0033] The invention provides a method for modulating an immune response comprising modulating a gene wherein the gene modulates a Th1 (by way of example but not limitation, auto-immune diseases) and/or Th2- (by way of example but not limitation, inflammatory diseases) mediated immune response. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance, for example, membrane C-type lectin-like homolog (EST AA914211: signature sequence OtS1-B7). C-type (Ca^{2+}-dependent) lectins represent an important recognition mechanism for oligosaccharides at cell surfaces, attached to circulating proteins and in the extra-cellular matrix. Binding of specific sugar structures by these lectins mediates biological events such as cell-cell adhesion, serum glycoprotein turnover and innate immune responses to potential pathogens. These proteins contain carbohydrate-recognition domains (CRDs) that mediate sugar binding. C-type lectins also contain a Ca^{2+} binding site. C-type lectins have been demonstrated to be present in antigen-presenting cells such as macrophages and dendritic cells. Interestingly, alveolar macrophages have been demonstrated to phagocytose allergens via an undefined C-type lectin leading to the induction of iNOS and subsequent generation of NO by alveolar macrophages. The NO generated by these macrophages may drive T-cell differentiation into the Th2 pathway by inhibition of Th1 responses. The invention provides a method for the treatment

and/or prevention of an immune-related response comprising providing the targeting of an antigen to this C-type lectin. This induces a Th2-dominated immune response and is effective in the treatment of Th1-mediated diseases such as auto-immune diseases.

[0034] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, a protein inhibitor of neuronal nitric oxide synthase (mPIN) (signature sequence R1-OS-B1-B1). Nitric oxide (NO) can be produced by several nitric oxide synthase enzymes (nNOS, iNOS and eNOS). Murine PIN is a cytoplasmic protein and is a selective inhibitor of neuronal nitric oxide synthase (nNOS). The human homolog appears to be dynein light chain 1 (hdlc1). NO has been implicated in several diseases including asthma and other inflammatory diseases. Interestingly, nNOS is located on chromosome 12q that has been linked to asthma. The invention shows a down-regulation of mPIN mRNA in lymph nodes of a mouse asthma model. NO negatively regulates type-1 T-helper lymphocyte (Th1) development. Likewise, NO may tip the balance between Th1 and Th2 cells in favor of Th2 responses. The invention provides a method for the treatment and/or prevention of an immune-related response, more particular Th2-mediated immune responses such as allergy and asthma comprising modulating PIN expression, more preferably, decreasing expression which leads to increased NO release and facilitation of Th2-mediated immune responses such as allergy and asthma. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising blockade of PIN activity which is beneficial in Th1-mediated diseases such as auto-immunity by increasing regulatory Th2 cells. Treatment with PIN is beneficial in Th2-mediated responses such as asthma and allergy by increasing regulatory Th1 cells. Besides a role of PIN in the regulation of T-cells, PIN plays a role in airway hyperresponsiveness. Neuronal NOS but not iNOS nor eNOS has been demonstrated to be crucial for baseline- and antigen-induced airway hyperresponsiveness in mice. Expression of nNOS but not eNOS nor

iNOS in airway epithelial cells of our mouse model of allergic asthma is demonstrated. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising modulating nNOS and PIN, more preferably, up-regulating nNOS in airway epithelial cells and down-regulating PIN. Up-regulation of nNOS in airway epithelial cells and a down-regulation of PIN can strongly potentiate the production of NO or its metabolites. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising modulating expression of PIN, more preferably, increasing expression of PIN which inhibits NO production by nNOS and inhibits airway hyperresponsiveness in asthma and related respiratory diseases associated with hyperresponsiveness such as COPD.

[0035] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, cathepsin B (signature sequence: OtS2-F2). Exogenous antigens are processed by lysosomal proteases within antigen-presenting cells to create antigenic peptides which are loaded into MHC class II molecules and expressed on the cell surface to $CD4^+$ T-lymphocytes. Enzymes such as aspartate proteases (e.g., cathepsin D and E) and cysteine proteases (e.g., cathepsin B, L and S) are proposed to be involved in this process. Interestingly, cathepsin B appears to be involved in the generation of Th2-dominated immune responses to ovalbumin and to a Leishmania infection in BALB/c mice. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising providing inhibition of the activity of cathepsin B by inhibitors. This inhibits allergic asthma and related allergic and Th2-mediated inflammatory responses.

[0036] Furthermore, the invention provides a method for the treatment and/or prevention of an immune-related response, comprising providing targeting of antigen to LR8 which will induce a Th2-dominated immune response and is effective in the treatment of Th1-mediated diseases such as auto-immune diseases. The invention provides a method for the

treatment and/or prevention of an immune-related response, comprising modulating Ly-GDI and/or Cdc42-GAP, more preferably, inducing the expression of these proteins. Modulating Ly-GDI and/or Cdc42-GAP, or inducing the expression of these proteins induces T-helper lymphocyte type-2 responses and is effective in the treatment of Th1-lymphocyte-mediated diseases like auto-immune diseases.

[0037] The invention provides a nucleic acid library comprising nucleic acid or functional fragments, derivatives or analogues thereof comprising genes as listed in Table 1, 2 or 3 which are implicated in oxidative stress responses and/or programmed cell death (PCD) (i.e., cellular apoptosis). The invention provides a method for treatment of an immune response wherein the nucleic acid is involved in the generation of anti-oxidants or free radicals. An "antioxidant" or "free radical scavenger" is an enzyme that prevents build up of reactive oxygen species (ROS) in cells. In general, antioxidants prevent tissue damage by oxidative stress. "Free radical generator" is an enzyme that is involved in the generation of ROS.

[0038] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, clusterin/Apolipoproteine J/sulphated glycoprotein 2 (signature sequence OtS2-B12). Clusterin is a 75-80 kDa disulphide-linked heterodimeric secreted glycoprotein. It is encoded by a single gene and the translated product is internally cleaved to produce its α and β subunits prior to secretion from the cell. It is ubiquitously expressed. There is extensive evidence of a correlation between clusterin expression and diseases, e.g., Alzheimer, glioma's or pathological stress. Many functions have been ascribed to clusterin such as controlling cell-cell and cell-substratum interactions; regulating apoptosis; transporting lipids; regulating complement and a general chaperone/heat-shock protein function.

[0039] The invention provides a method for the treatment and/or prevention of an immune-related response, comprising modulating clusterin, more preferably, increasing the

expression of clusterin, which will inhibit allergic asthma and related allergic and inflammatory diseases.

[0040] Moreover, antioxidants may inhibit the expression of genes regulated by the "redox status" within inflammatory cells, such as the ras pathway. Oxidative stress also appears to be involved in the activation of the CD4-associated protein tyrosine kinase $p56^{lck}$. $P56^{lck}$ is an important protein in the activation of $CD4^+$ T-lymphocytes. Oxidative stress is increased in patients with asthma and chronic obstructive pulmonary disease (COPD) and it is possible that reactive oxygen species contribute to its pathophysiology. Likewise, antioxidants might be of use in the therapy of these respiratory diseases. Oxidative stress has also been shown to regulate the cellular glucocorticoid responsiveness. A decreased sensitivity to glucocorticoids has been observed in patients with allergic asthma leading to treatment with either high-doses of glucocorticoids or inappropriate treatment. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention provides various anti-oxidant proteins down-regulated upon OVA challenge in the mouse asthma model, e.g., Selenoprotein P (signature sequence: R1-OS-B1-H1), Gluthation-S-transferase mu2 (signature sequence: OtS2-E6), Ferritine (signature sequence: R1-OS-B1-O5), Anti-oxidant protein 2 (signature sequence: OtS2-A6).

[0041] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, selenium. Selenium is an essential trace element that is incorporated as selenocysteine into the primary structure of selenoproteins. There are at least ten animal selenoproteins. Animal studies have demonstrated a role for selenium in oxidant defense, thyroid hormone metabolism, and defense against viral infections. Selenoproteins presumably

mediate these biologic effects. Most of the human selenoproteins are members of the gluthatione peroxidase or iodothyronine deiodinase families. Selenoprotein P (SEPP1) is not a member of these families. It is an extracellular glycoprotein that is present in several isoforms and is the only selenoprotein known to contain multiple selenocysteine residues. It is a heparin-binding protein that appears to be associated with endothelial cells and has been implicated as an oxidant defense in the extracellular space. There is evidence that several isoforms of the protein exist, likely products of the same gene. Human selenoprotein has been mapped to chromosome 5q31. Interestingly, many studies have demonstrated a linkage between chromosome 5q and allergy, asthma and airway hyperreactivity. There is considerable evidence that oxidative stress is increased in patients with chronic obstructive pulmonary disease (COPD) and that reactive oxygen species contribute to its pathophysiology. Likewise, it has been postulated that antioxidants might be of use in the therapy of COPD. Selenoprotein P may be useful as a therapeutic protein in diseases that are associated with increased oxidative stress such as COPD, asthma and other inflammatory diseases. It was observed that mRNA levels of selenoprotein P are decreased in lymph node tissue of a mouse asthma model. Selenium and selenoproteins have been shown to play a role in the function of granulocytes and lymphocytes. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising modulating selenoprotein P.

[0042] The invention provides a method for modulating an immune-related response, comprising modulating the generation of antioxidants or free radicals. Treatment with antioxidant proteins (e.g., by inhalation) or induction of the expression of these proteins and/or suppression of free radical generators in airway tissue can be used to treat allergic inflammation or related inflammatory diseases or diseases associated with increased oxidative stress such as asthma and COPD. Treatment with antioxidant proteins or induction of the expression of these proteins in airway tissue together with glucocorticoid treatment can limit the dose of glucocorticoids required for a therapeutic effect in patients with allergic asthma and other chronic inflammatory diseases associated with glucocorticoid insensitivity.

[0043] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the

production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, cytochrome P-450 naphthalene hydroxylase (CYP2F2) enzymes (signature sequence R1-OS-B1-A1). CYP2F2 are a superfamily of more than 160 known members that play a major role in the metabolism of numerous physiological substrates and a wide array of xenobiotics including drugs, chemical carcinogens, insecticides, petroleum products, and other environmental pollutants. Oxidative metabolism catalyzed by cytochrome P450s can result in detoxification. In some instances it results in metabolic activation of a chemical to cytotoxic and/or carcinogenic forms. Although the liver is the primary organ for drug metabolism, extrahepatic tissues such as lung, kidney and intestine, also play an important role in detoxification or biotransformation of xenobiotics. Each tissue has a unique P450 isozyme distribution and regulatory mechanism for cytochrome P450 gene expression. Currently, the members of the CYP2F gene subfamily that are selectively expressed in lung tissues consist of human CYP2F1 and mouse CYP2F2 and CYP2F3. Human CYP2F1 bioactivates 3-methylindole, while mouse CYP2F2 bioactivates naphthalene Mouse CYP2F3 catalyzes the dehydrogenation of 3-methylindole but not its hydroxylation. Murine CYP2F2 is expressed in lung tissue as well as in liver. In the lung, it plays an important role in the metabolic activation of substrates that cause lung injury. CYP2F2 is involved in the hydroxylation of naphthalene and it specifically catalyzes the production of a very reactive and potentially toxic intermediate, the 2R, 2S arene oxide, that is associated with necrosis of unciliated bronchiolar epithelial cells or CLARA cells in lung tissue. Several P450 enzymes with epoxygenase activity have also been shown to be involved in the metabolism of arachidonic acid into biologically active eicosanoids. Based on the bioactivation of naphthalene, we anticipate that CYP2F enzymes also display epoxygenase activity. The epoxygenase pathway leads to the formation of four regio-isomeric epoxy-eicosatrienoic acids (EETs): 14,15-EET, 11,12-EET, 8,9-EET and 5,6-EET. From these epoxides, other lipid mediators can be generated such as 14,15-DHET, 11,12-DHET, 8,9-DHET, 5,6-DHET and 5,6-epoxy prostaglandin E1. Some of these epoxides have been shown to induce vasorelaxation. 5,6-EET and 11,12-EET have also

been shown to modulate tracheal chloride-channel activity and induce airway smooth muscle relaxation. Epoxides generated through CYP2F may, therefore, protect against excessive bronchoconstriction and may be involved in airway hyperreactivity in asthma and other respiratory diseases. Epoxygenase metabolites have also been shown to have anti-inflammatory activities such as inhibition of leukocyte adhesion to the vascular wall and inhibition of IκB kinase thereby preventing the activation of NF-κB. Cytochrome P-450 naphthalene hydroxylase (CYP2F2). A strong (>ten-fold) down-regulation of cytochrome P450 (CYP2F2) mRNA in a mouse asthma model in the lymph nodes of "asthmatic" (OVA-challenged) compared to "healthy" (saline-challenged) mice was observed. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising modulating the expression of CYP2F, more preferably, increasing expression of CYP2F in airway tissue and/or by preventing its down-regulation. This inhibits airway hyperresponsiveness and excessive bronchoconstriction and can be used to treat allergic asthma and other respiratory diseases associated with hyperresponsiveness such as COPD. The invention provides a method for the treatment and/or prevention of an immune-related response, comprising providing local treatment (inhalation) with CYP2F metabolites of arachidonic acid, in particular 11,12-EET, which inhibits airway inflammation for treatment of allergic asthma and other respiratory inflammatory diseases such as COPD. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising modulating the enzymatic activity of CYF2F, more preferably, stimulating the enzymatic activity of CYF2F by an allosteric stimulator which increases the generation of epoxides and likewise inhibits airway hyperresponsiveness and airway inflammation. Stimulation of the enzymatic activity of CYF2F by an allosteric stimulator is effective in the treatment of allergic asthma and other respiratory diseases such as COPD.

[0044] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar

manifestations of asthma. For example, four families of structurally related heat-shock proteins are distinguished based on their molecular weights: Hsp90, Hsp70, Hsp60 and small Hsps. By definition, Hsp expression is elevated in cells undergoing stress, such as those in damaged or inflamed tissue. Conditions as diverse as a rise in temperature, hypoxia, irradiation, infection and exposure to toxic chemicals can all result in increased Hsp expression. Heat-shock cognate protein (Hsc)73 is a constitutively expressed member of the Hsp70 family. Hsc73 is expressed in the cytosol but is also present in lysosomes. Hsc73 plays a role in binding and protecting peptides from extensive degradation and facilitating the kinetics of peptide transfer to MHC class II molecules. Hsc73 is also present in dendritic cell-derived exosomes which have been shown to elicit potent T-cell-dependent immune responses in mice. Moreover, a receptor for Hsp70 proteins is present on the surface of macrophages and dendritic cells and Hsp70 can induce macrophages to activate T-cells independently of antigen. Thus, Hsc73 appears to be involved in antigen-presentation and T-cell activation. Administration of antigen or antigenic peptides together with Hsp70 proteins has been shown to generate $CD8^+$ T-lymphocyte responses when administered to laboratory animals. Moreover, Hsp70 is involved in cross-priming of $CD8^+$ cells by APC upon antigen processing. Recently, Hsp70 has also been shown to be involved in the induction of regulatory T-cells. Hsc73 (signature sequence: OtS2-H2) may also be involved in the induction of inducible nitric oxide synthase (iNOS) by LPS or cytokines via an effect on p38 mitogen-activated protein (MAP) kinase. In agreement herewith, the selective hsc73 inhibitor deoxyspergualin inhibits the induction of iNOS by cytokine- or endotoxin-activated macrophages. NO has been shown to inhibit the generation of Th1 lymphocytes thereby tipping the balance towards Th2 immune responses. In airway epithelial cells, Hsp70 has been shown to have potent anti-inflammatory effects by stabilization of IκBα through preventing the activation of IκB kinase leading to inhibition of NF-κB activation and down-stream gene transcription. In airway epithelial cells, increased Hsp70 expression suppressed cytokine-induced expression of pro-inflammatory cytokines IL8 and TNFα.

[0045] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the

antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against Hsc73. This inhibits the generation of NO by APCs and thereby limits a Th2-dominated immune response by increasing Th1 immunity. This treatment is effective in the treatment of allergic asthma and related allergic and inflammatory responses.

[0046] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, more particularly allergic inflammation or related inflammatory diseases (e.g., COPD) comprising modulating, more preferably, up-regulating the expression of Hsc73 leading to induction and/or elevation of the expression of Hsc73 protein in airway epithelial cells.

[0047] The invention provides a method for treatment of an immune response comprising providing an antagonist of antigen processing and presentation. "Antagonist" herein refers to a molecule that bears sufficient structural similarity to a second molecule to compete with that molecule for binding sites on a third molecule, such as, for example, an antibody. An "antibody" herein refers to a protein produced by lymphoid cells in response to foreign substances (antigens) and capable of coupling specifically with the antibody's homologous antigen (i.e., the one that stimulated the immune response) or with substances that are chemically very similar to that antigen. "Antibody" herein refers to both polyclonal and monoclonal antibodies.

[0048] The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance; by way of example, the invention provides nucleic acids as listed in Table 1, 2 or 3 which are involved in antigen processing and presentation, such as, MHC-II (signature sequence: StO1-B5), H2-Oa (signature sequence: SvO2-1-A4), EST: Clathrin (signature sequence: SvO2-1-D4), Aspartyl aminopeptidase (signature sequence: StO1-c1), Cathepsin B (signature sequence: OtS2-F2), Breast heat shock 73 protein (signature sequence: OtS2-H2), EST: C-type lectin (signature sequence: OtS1-B7), Ubiquitin-specific protease (signature sequence: R1-OSB1-A2), Ubiquitin/60s (signature sequence: SVO2-1-C12) and Lysozyme M (OtS2-B1). Antigen-presenting cells play an important role in the differentiation of $CD4^+$ and $CD8^+$ T-lymphocytes into particular subsets (Type-1, Type-2, Type-3 or regulatory types) and are important for the generation of either a detrimental or a beneficial immune response to antigens.

[0049] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, Phospholipase Cγ2 (PLCγ2) (signature sequence: SvO2-1-A8). PLCγ2 unlike PLCγ1 which is expressed in many cell types, PLCγ2 is only expressed in hematopoietic cells (e.g., B-lymphocytes, NK-cells, platelets, granulocytes, monocytes/macrophages and mast cells). PLCγ2 is a cell signaling molecule with many regulatory domains, e.g., SH2, SH3, and pH domains. It catalyzes the hydrolysis of phosphatidyl-inositol 4,5-bisphosphate to yield the

second messengers, IP3 and DAG. PLCγ2 has been shown to be involved in production of reactive oxygen intermediates by neutrophils. In addition to PLCγ1, PLCγ2 is activated upon triggering of mast cells via Fcε RI. The promoter region of PLCγ2 has Sp1, NF1, AP2, SRE, EBF and CACCC box consensus sites. In B-cells, mRNA expression of PLCγ2 is enhanced by serum, TPA, retinoic acid and 5-azacytidine. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against PLCγ2 or a proteinaceous substance comprising PLCγ2 which is effective in the treatment of allergic asthma and related allergic and inflammatory diseases.

[0050] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example,

APLP2 C31 (signature sequence: SvO2-1-B7) peptide is involved in cell death (apoptosis). Apoptosis or cell death is an important mechanism to limit immune reactions. The cytoplasmic domain of APLP2 containing the "NPTY" motif (SEQ ID NO: 2) is involved in T-lymphocyte activation upon phosphorylation of the tyrosine (Y) residue leading to Shc binding. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against APLP2, more specifically, the cytoplasmic domain of APLP2 containing the "NPTY" motif (SEQ ID NO: 2). This prevents the Ras-pathway of T-lymphocyte activation and inhibits an immune response and is effective in the treatment of allergic asthma and related allergic and inflammatory diseases. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against "VEVD" (SEQ ID NO: 3) and "NPTY" motif (SEQ ID NO: 2) inhibits unwanted cell death mediated by this pathway and is effective in the treatment of allergic asthma and related allergic and inflammatory diseases. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the

production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing inhibition of the generation of the C-terminal 31 amino acid APLP2 peptide by caspases and/or proteases encoded by the nucleic acid of Table 1, 2 or 3 which inhibits unwanted cell death mediated by this pathway.

[0051] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against hCaCC1 (or gob-5) (signature sequence: R1-SO-R1-C11) which inhibits mast cell activation and can be used in the treatment of immune diseases in which mast cells play an important role such as all allergic diseases (rhinitis, atopic dermatitis, asthma, urticaria) and auto-immune diseases (e.g., multiple sclerosis).

[0052] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as

shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Table 1, 2 or 3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against Hsc73. This inhibits the generation of NO by APCs and thereby limits a Th2-dominated immune response by increasing Th1 immunity. This treatment is effective in the treatment of allergic asthma and related allergic and inflammatory responses.

[0053] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against LR8 which inhibits allergic asthma and related allergic and inflammatory diseases.

[0054] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as

shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. More preferably, a method of treatment and/or prevention of an immune-related response, even more preferably, allergic asthma and related allergic and inflammatory diseases, comprising providing an antagonist(s) directed against one or more up-regulated genes as listed in Table 1, 2 or 3 or the corresponding proteinaceous substances.

[0055] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against Ly-GDI (signature sequence: SVO2-1-D8) and/or Cdc42-GAP (signature sequence: R1-SO-R1-A12) which inhibits T-helper lymphocyte type-2 (Th2) responses and is effective in the treatment of allergic asthma and related allergic and Th2-mediated inflammatory diseases.

[0056] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic

acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against C-type lectin (signature sequence: Ot-S2-B7) which inhibits antigen presentation and skewing towards a Th2-dominated immune response. This blockade is effective in the treatment of allergic asthma and related allergic and inflammatory diseases.

[0057] The invention provides a method for modulating an immune response wherein a gene modulates $CD8^+$ T-lymphocyte responses. Also provided is a gene or gene product capable of inducing a specific regulatory $CD4^+$ and/or $CD8^+$ T-lymphocyte response that inhibits Th2-dominated allergic responses. The invention provides a method for modulating an immune response wherein the gene modulates $CD4^+$ T-lymphocyte responses. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma, for example, Ubiquitin-specific protease (UBP43)(signature sequence R1-OS-B1-A2). UBP43 belongs to a family of ubiquitin-specific proteases (UBP) and has a molecular mass of 43 kDa. Protein ubiquitination has been implicated in many important cellular events. The human homolog of this protein is ISG43. In wild-type adult mice, UBP43 is

highly expressed in thymus and peritoneal macrophages. Furthermore, it is expressed in cell lines of the monocytic lineage and its expression is regulated during cytokine-induced monocytic cell differentiation. Over expression of UBP43 has been shown to block cytokine-induced terminal differentiation of the monocytic cell line M1. Down-regulation of UBP43 mRNA in lymph nodes of a mouse asthma model was observed. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising modulating the expression of UBP43, more preferably, increasing the expression of UBP43 in APCs which prevents allergic asthma and related respiratory disease by increasing the generation of regulatory $CD8^+$ T-lymphocytes. The proteasome is involved in the generation of MHC class-I peptides by proteases.

[0058] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance. The invention further provides the use of the antagonist such as an antibody directed against a proteinaceous substance derived from at least a nucleic acid as shown in Tables 1-3 for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma. For example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing inhibition of peptide loading into MHC class-I molecules by proteases encoded by the nucleic acid as outlined in Table 1, 2 or 3, which inhibits the generation of $CD8^+$ T-lymphocyte responses (i.e., T-lymphocyte costimulation). Airway wall remodeling is an established pathological feature of asthma but its causes are not well understood. One cytokine of potential relevance is transforming growth factor-beta1 (TGF-beta 1). In patients with asthma, matrix-associated TGF-beta 1 is likely to be bound at least in part to decorin (signature sequence: R1-OS-B1-C5). This interaction may provide a reservoir of TGF-beta 1 that can be released in an active form in response to appropriate stimuli. Decorin is also a natural inhibitor of TGF-beta

and has been shown to restore T-lymphocyte responses to mycobacteria. The invention provides for a method of treatment and/or prevention of an immune-related response, comprising modulating the expression of decorin, preferably, increasing the expression of decorin. Increased expression of decorin in airway tissue and/or treatment (inhalation) with decorin inhibits the effects on TGF-beta on airway tissue remodeling and is effective in the treatment of immune-related responses.

[0059] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance; for example, the invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing immunotherapy using Hsc73, alone or together with an antigen/allergen. An "allergen" herein is defined as a substance inducing hypersensitivity. Immunotherapy using Hsc73, alone or together with an antigen/allergen induces a specific regulatory $CD4^+$ or $CD8^+$ T-lymphocyte response that inhibits Th2-dominated allergic responses.

[0060] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance; for example, the invention provides a method for modulating an immune response of an individual wherein a gene encodes a gene product capable of modulating an immune response. A "gene product" herein refers to the mRNA and the polypeptide chain translated from an mRNA molecule, which in turn is transcribed from a gene; if the RNA transcript is not translated

(e.g., rRNA, tRNA) the RNA molecule represents the gene product. The gene product herein can refer to any proteinaceous substance. A proteinaceous substance can refer to any molecule comprising amino acid and/or peptide or protein.

[0061] The invention provides alleles of the polypeptide(s) encoded by nucleic acid sequences of this invention. As used herein, an "allele" or "allelic sequence" is an alternative form of the polypeptides described above. Alleles result from a mutation (e.g., a change in the nucleic acid sequence, and generally produce altered mRNA or a polypeptide whose structure or function may or may not be altered). Any given polypeptide may have none, or more allelic forms. Common allelic changes that give rise to alleles are generally ascribed to natural deletions, additions or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence. Deliberate amino acid substitution may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathetic nature of the residues as long as the biological activity of the polypeptide is retained. Altered nucleic acid sequences of this invention include deletions, insertions, substitutions of different nucleotides resulting in the polynucleotides that encode the same or are functionally equivalent. A "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent. An "insertion" or "addition" is that change in nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring polypeptide(s). A "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. The invention includes variants of the polypeptide. A "variant" of a polypeptide is defined as an amino acid sequence that is different by one or more amino acid substitutions. A variant may have conservative changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely a variant may have nonconservative changes (e.g., replacement of a glycine with a tryptophan). Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted, without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNAStar software.

[0062] The invention provides a method of modulating an immune response wherein the immune response comprises airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma.

[0063] The invention provides a method modulating an immune response wherein a gene is modulated by transducing a cell of an individual. Methods to transduce cells are known in the art. Target cells can be transduced with a nucleic acid delivery vehicle comprising at least one nucleic acid of the invention. A "gene delivery vehicle" herein is used as a term for a recombinant virus particle or the nucleic acid within such a particle, or the vector itself, wherein the vector comprises the nucleic acid to be delivered to the target cell(s) and is further provided with a means to enter the cell(s). This cell(s) can be used for drug screening and drug discovery.

[0064] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance; for example, the invention provides a substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3.

[0065] A "substance" herein refers to any material entity capable of modulating a gene of the invention, for example, an "entity" can be a molecule wherein the molecule is a chemical compound. The substance can also be an antigen, such as a foreign invader comprising a protein or protein attached moiety. The substance can also be of proteinaceous origin comprising amino acid and/or peptide or protein.

[0066] The invention provides a medicament comprising a substance capable of modulating a gene(s) of the invention. A preferred embodiment is a medicament which is a pharmaceutical. Suitable pharmaceutical compositions are known.

[0067] The invention provides the use of a substance for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma.

[0068] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Tables 1-3 and use of the substance for the production of an antagonist against substance; for example, the invention provides the use of a proteinaceous substance derived from a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 for the production of an antagonist against the substance. "Antagonist" herein refers to a molecule that bears sufficient structural similarity to a second molecule to compete with that molecule for binding sites on a third molecule, for example, an antibody.

[0069] The invention provides the use of a proteinaceous substance derived from a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3 for the production of an antagonist against the substance, wherein the antagonist is an antibody or functional equivalent thereof. An "antibody" herein refers to a protein produced by cells in response to foreign substances (antigens) and capable of coupling specifically with its homologous antigen (i.e., the one that stimulated the immune response) or with substances that are chemically very similar to that antigen. Antibody herein refers to both polyclonal and monoclonal antibodies.

[0070] The invention provides for a method of treatment and/or prevention of an immune-related response, comprising providing an antagonist(s) directed against a proteinaceous substance derived from a nucleic acid sequence at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. The invention provides a substance such as a proteinaceous substance capable of modulating a gene comprising a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as

shown in Tables 1-3 and use of the substance for the production of an antagonist against the substance; for example, the invention provides an antagonist directed against a proteinaceous substance derived from a nucleic acid at least functionally equivalent to a nucleic acid identifiable by a signature sequence as shown in Table 1, 2 or 3. "Functionally equivalent" herein means that the subject signature sequence can vary from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which will not result in a functional dissimilarity between the two sequences.

[0071] The invention provides an antagonist comprising an antibody or functional equivalent thereof. An antibody or functional equivalent thereof can refer to synthetic molecules (i.e., antibodies derived by chemical synthesis) and encompasses all molecules capable of coupling with proteinaceous substance(s) derived from a nucleic acid of the invention. Proteinaceous substance herein can refer to an entity derived from the nucleic acids of the invention capable of modulating an immune response.

[0072] The invention provides a medicament comprising an antagonist. The invention provides the use of an antagonist for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma.

[0073] The invention provides for use of an antagonist for the production of a medicament for the treatment of an immune response observed with airway hyperresponsiveness and/or bronchoalveolar manifestations of asthma.

[0074] The invention further provides a diagnostic kit for screening for an immune response comprising providing a nucleic acid the subject of the invention. Methods of screening are known in the art. These procedures include, but are not limited to, DNA-DNA, DNA-RNA hybridization. The form of such quantitative methods may include Southern or Northern analysis, dot/slot blot or other membrane based technologies; PCR technologies such as DNA Chip, Taqman®, NASBA, SDA, TMA, *in situ* hybridization, protein bioassay or immunoassay techniques ELISA, IFA, proteomic and metabolic technologies.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0075] FIG. 1. Effects of Ly-GDI and Cdc42-GAP on small GTP-binding proteins Rac and Cdc42.

[0076] FIG. 2. PCR products using cDNA obtained from dorsal root ganglia (DRG) isolated from "healthy" or "asthmatic" mice. PCR was carried out using conditions well known in the art using the gene-specific primer pairs for:

a) EST AA726662 (Top) (sense primer: GGTTGAGGAGCGGAATGGAAGAGC (SEQ ID NO: 22); antisense primer: ATTGCCCACGGCGCTATCCA, (SEQ ID NO: 23) product length 362 base pairs);

b) m_CaCC (Middle) (sense primer: ATTAGTCACATTTGACAGCGCTGCC (SEQ ID NO: 26); antisense primer: TGGGAGACGCTGCCACTTGTAGAT, (SEQ ID NO: 27) product length 414 base-pairs); and for

c) gob 5 (Bottom) (sense primer: GCCTTCGGACAGCATTTACA, SEQ ID NO: 4; anti-sense primer TGCGTTGTCCAGGTGATAAG, SEQ ID NO: 5; product length 435 base-pairs).

Lane 1 refers to 100 bp DNA ladder; lanes 2, 4 and 6 refer to cDNA obtained from DRG of "healthy" mice and prediluted respectively 1/4, 1/16 and 1/32; lane 3, 5 and 7 refers to cDNA obtained from DRG of "asthmatic" mice and prediluted respectively 1/4, 1/16 and 1/32.

[0077] FIG. 3. Homology between mouse LR8 (SEQ ID NO: 125), human LR8 (SEQ ID NO: 126), and the beta chain of the high affinity mouse IgE receptor (SEQ ID NO: 123) and the beta chain of the high affinity human IgE receptor (SEQ ID NO:124).

[0078] FIG. 4. Genes down-regulated in "asthma" versus "healthy" mice, Sequence R1-OS-B1-E5 (SEQ ID NO: 127) and Sequence OtS2-C5 (SEQ ID NO: 128).

[0079] FIG. 5A. PCR analysis of gene-fragments with signature sequences R1-OS-B1-C3 and OtS2-C5 using cDNA from dorsal root ganglia obtained from saline- (SAL) or ovalbumin- (OVA) challenged mice as described in Example 1. HPRT house-keeping control gene is used to control for the relative amount of cDNA. Two-fold dilution series from left to right. The black bars indicate the dilutions that gave a PCR product.

[0080] FIG. 5B. PCR analysis of genes Cyp2f2 and Gob-5 using cDNA from lung tissue obtained from saline- (SAL) or ovalbumin- (OVA) challenged mice as described in

Example 1. HPRT house-keeping control gene is used to control for the relative amount of cDNA. Two-fold dilution series from left to right. The black bars indicate the dilutions that gave a PCR product.

[0081] FIG. 6. Gene comprising OtS1-B7 fragment.

[0082] FIG. 7. Restriction map of Contig 1A, the gene comprising OtS1-B7 fragment.

[0083] FIG. 8. EtBr-staining of the restriction-digests used (panel A), the autoradiograph after two and five days' exposure time (panels B and C) and the interpretation (panels D and E). Panel D shows a graphical representation of all the hybridizing bands, the thickness of the bands indicates their relative strengths as judged by eye using both exposures. Panel E shows the expected hybridization pattern based on the predicted restriction enzyme map (shown in FIG. 3). The thickness of the bands is drawn proportional to the length of the hybridizing region present in each restriction fragment.

[0084] FIG. 9. Complete sequence of Contig 1A, SEQ ID NO: 129, the gene comprising OtS1-B7 fragment. Contig1A consists of the following overlapping contigs present in Genbank acc. AC073804 and AC73706:

nt 1-11054 = nt 294022-305082 from AC073804

nt 11009-19619 = nt 237022-228395 from AC073804 (reverse complement)

nt 1805-7790 = nt 39946-34025 from AC073706 (reverse complement)

nt 6918-15759 = nt 32026-23233 from AC073706 (reverse complement)

[0085] FIG. 10. The protein (SEQ ID NO: 130, 325 AA) encoded by the predicted gene encompassing Contig 1A comprising the OtS1-B7 fragment.

[0086] FIG. 11. CLUSTAL W (1.81) multiple sequence alignment of the polypeptide derived from the gene comprising OtS1-B7 (OtS1-B7-ORF, SEQ ID NO: 130) and DC-SIGN (GenBank acc.nr. AAF77072, SEQ ID NO: 131), also designated CD209.

[0087] FIG. 12. Outline for the generation of a genetically engineered null-mice for murine DC-SIGN (signature sequence OtS1-B7, indicated as OtB7 in the figure), the mouse homolog of human DC-SIGN.

[0088] FIG. 13. Lanes 1-8 represent PCR products obtained using nonstimulated human lung epithelial cell line H292. Lanes 10-17 represent PCR products obtained using PMA

(10 ng/ml for three hours) stimulated H292 cells. Lanes 19-26 represent PCR products obtained using IL-9 (U/ml for three hours) stimulated H292 cells.

PCR products in lanes 1, 2, 10, 11, 19, 20 represent housekeeping enzyme HPRT.

PCR products in lanes 3, 4, 12, 13, 21, 22 represent Calcium activated Chloride Channel 1 (CLCA1).

PCR products in lanes 5, 6, 14, 15, 23, 24 represent CLCA4

PCR products in lanes 7, 8, 16, 17, 25, 26 represent CLCA2

Lanes 9, 18, 27 represent a 100 bp ladder.

[0089] FIG. 14. ClustalW analysis of calcium-activated chloride channels, including Gob-5 (SEQ ID NO: 132), HuCLCA1 (SEQ ID NO: 133), HuCLCA4 (SEQ ID NO: 134), MuCaCC (SEQ ID NO: 134) and HuCLCA2 (SEQ ID NO: 136). Indicated are conserved cysteines, the von Willebrand factor type A domain and the MIDAS motif. CLUSTAL W (1.81) multiple sequence alignment.

[0090] FIG. 15. ClustalW analysis of CD59 (SEQ ID NO: 139), signature sequence OtS2-D10 (SEQ ID NO: 138) and EST (GenBank acc. BE655906, SEQ ID NO: 137). Indicated are the forward and reverse primers as described in Table 1 (example 1). CLUSTAL W (1.81) multiple sequence alignment.

[0091] FIG. 16. Top: Schematic representation of APLP2 mRNA and protein with the KPI domain (exon 7) and the slected primer pair to identify splice variants with or without the KPI-domain. Bottom: PCR analysis of cDNA from dorsal root ganglia obtained from saline- (SAL) or ovalbumin- (OVA) challenged mice as described in Example 1). HPRT house-keeping control gene is used to control for the relative amount of DNA. The black bars indicate the dilutions that gave a PCR product.

[0092] FIG. 17. PCR analysis of murine Plunc (signature sequence R1-OS-B1-D6) of cDNA from dorsal root ganglia obtained from saline- (SAL) or ovalbumin- (OVA) challenged mice as described in Example (1). HPRT house-keeping control gene is used to control for the relative amount of DNA. Two-fold dilution series from left to right. The black bars indicate the dilutions that gave a PCR product.

[0093] FIG. 18A. Airway responsiveness to aerosolized methacholine was measured in conscious, unrestrained mice (group I) 24 hours after the third OVA inhalation challenge.

Airway responsiveness in control mice (first and second bar) and ERTR9 treated mice (third and fourth bar) was measured before (plain bars) and after (striped bars) the OVA aerosol challenge period. Values are expressed as mean ± SEM. Post challenge airway responsiveness is significantly increased in control mice. * $P<0.05$ as determined by the student's t- test.

[0094] FIG. 18B. Airway responsiveness to aerosolized methacholine was measured in conscious, unrestrained mice (group II) 24 hours after the third OVA inhalation challenge. Airway responsiveness in control mice (first and second bar) and ERTR9 treated mice (third and fourth bar) was measured before (plain bars) and after (striped bars) the OVA aerosol challenge period. Values are expressed as mean ± SEM. Post challenge airway responsiveness is significantly reduced in ERTR9-treated mice compared to controls. * $P<0.05$ as determined by the student's t- test.

[0095] FIG. 19A. Cellular composition of the BALF was determined 24 hours after the third OVA inhalation challenge. Control mice (white bars) and ERTR9 treated mice (grey bars). Values are expressed as mean ± SEM. *$P<0.05$ as determined by the student's t-test. Eo: eosinophils; neutro: neutrophils; MNC: mononuclear cells.

[0096] FIG. 19B. Cellular composition of the BALF was determined 24 hours after the third OVA inhalation challenge. Control mice (white bars) and ERTR9 treated mice (grey bars). Values are expressed as mean ± SEM. Eo: eosinophils; neutro: neutrophils; MNC: mononuclear cells.

DETAILED DESCRIPTION OF THE INVENTION

EXAMPLES

Example 1: Development of murine model of allergic asthma

[0097] Due to the limitations of experimental studies in patients with allergic asthma a murine model with immunologic and pathophysiologic features reminiscent of allergic asthma was developed (A.J. Oosterhout (1998)*: Am J Respir Cell Mol Biol*; 19:826-35). There are several advantages to using a murine model compared to using tissues obtained from asthma patients such as (i) availability of isolated tissues or cells (ii) genetic homogeneity, (iii) identical age, (iv) well-controlled environment (food, specified pathogens, climate), and (v) ability to do time-series experiments (i.e., induction vs effector phase). In this model, Balb/c mice are

sensitized with ovalbumin (OVA) and repeatedly challenged by inhalation of OVA aerosol. This model is characterized by the presence of OVA-specific IgE antibodies in serum, airway eosinophilia and nonspecific hyperresponsiveness concomitant with the appearance of Th2-like cells in lung tissue and lung draining (thoracic) lymph nodes.

Example 2: Representational Difference Analysis (RDA)

[0098] Representational Difference Analysis of cDNAs (RDA) was employed to identify novel key regulatory molecules involved in the initiation and/or progression and/or suppression and/or repression of asthma symptoms. RDA analysis was performed according to previously defined methods (Groot and van Oost (1998), *Nucleic Acids Res:* 26:4476-81; Welford *et al.*, (1998): *Nucleic Acids Res.* 1998; 26:3059-65; Geng et al., (1998): *Biotechniques* 25:434-8). Gene expression between lung-draining lymph nodes (containing amongst others dendritic cells, macrophages, B- and T-lymphocytes, mast cells) obtained from "healthy" control animals and those obtained from "asthmatic" mice that display airway manifestations of asthma such as airway hyperresponsiveness and bronchoalveolar eosinophilia were compared. Balb/c mice were intraperitoneally sensitized with ovalbumin and later on repeated challenged by inhalation of saline aerosol (control or "healthy" animals) or ovalbumin aerosol ("asthmatic"). Lymph nodes were isolated at six hours after the last challenge. Using RDA, differentially expressed gene fragments were identified. Up-regulated genes are those that are expressed at higher levels in asthmatic tissue compared to healthy tissue. Vice versa, down-regulated genes are those that are expressed at lower levels in asthmatic tissue compared to healthy tissue. NCBI (National Center of Biotechnology Information) BLAST searches with the differentially expressed gene fragments against publicly available databases revealed significant alignment with either known genes (human or mouse), with expressed sequence tags (ESTs) or in some cases did not reveal a significant alignment or an incomplete alignment (unknown genes). The identified differentially expressed genes are listed in Table 1.

Example 3: Microarray experiment

[0099] Detection of differentially expressed genes in "asthmatic" mice compared with "healthy" control animals was performed using representational differences analysis coupled to

microarray hybridization methods as described previously (Welford *et al.,* (1998), *Nucleic Acids Res:* 26:3059-65). Unique differentially expressed genes (tethered nucleic acid: target) obtained from the RDA experiment (Example 2) were amplified by PCR using M13 primers, precipitated and spotted (arrayed in duplicate) onto chemically-modified glass slides (Corning) using a robotic printing device. Messenger RNA obtained from both lymph nodes of "healthy" and from "asthmatic" mice was transcribed into double-stranded cDNA and amplicons were generated. Amplicons were subsequently fluorescently labeled with either cyanine 3 (Cy3-ULS) or cyanine 5 (Cy5-ULS) dyes (i.e., one mRNA population (probe: free nucleic acid) was labeled with cyanine 3 (Cy3-ULS) and the other with cyanine 5 (Cy5-ULS)). The labeled probes (free nucleic acids) were then mixed and hybridized simultaneously to a microarray. The microarray was hybridized with both the Cy3 and Cy5 labeled probes in order to determine the expression ratio between both samples. After hybridization, the fluorescence pattern of each microarray was recorded for the Cy3 and Cy5 fluorescent dyes. Detailed statistical analyses were applied in order to determine the minimal significant ratio in each experiment. Clones that exhibited differential fluorescence were identified. In Table 1, the expression ratio ("asthma" : "healthy") is given.

Example 4: Virtual Northern Blot

[00100] Messenger RNA obtained from lymph nodes of "healthy" and "asthmatic" mice was transcribed into double-stranded cDNA and amplicons were generated. Using agarose gel electrophoresis, different amounts of amplicons were run and subsequently blotted onto Hybond filter membrane. Specific and individual gene fragments obtained by RDA from the lymph nodes of "healthy" and "asthmatic" mice were subcloned and subsequently amplified using M13 primers and fluorescently labeled (by random primer labeling). Labeled gene fragments were hybridized on the filter membrane containing the blotted amplicons and analyzed by a fluor-imager. After hybridization, based on the fluorescence intensity between amplicons obtained from "healthy" and "asthmatic" mice, an expression ratio ("asthma": "healthy") was determined (Table 1).

Example 5: By way of example, one novel therapeutic target protein for the treatment of immune and/or inflammatory responses

[00101] The mRNA expression of gob 5 has been examined by PCR using gene-specific primer pairs (sense primer: GCCTTCGGACAGCATTTACA (SEQ ID NO: 4); anti-sense primer TGCGTTGTCCAGGTGATAAG (SEQ ID NO: 5); product length 435 base-pairs). Gob 5 mRNA is present in lymph nodes, lung tissue, bronchoalveolar lavage cells and bone marrow obtained from healthy BALB/c mice. In tissues obtained from "asthmatic" mice compared to tissues obtained from "healthy" mice, the expression of gob 5 mRNA is increased in lymph nodes (approximately four-fold), bronchoalveolar lavage cells (>ten-fold), and bone marrow cells (approximately two-fold). Mucus secreting goblet cells have never been described in lymph nodes or bone marrow. The expression of gob 5 in murine bone marrow-derived mast cells and murine mast-cell lines is demonstrated (P815 and CFTL-12). Additionally, a strong up-regulation of gob 5 in the dorsal root ganglia (DRG) obtained from the mouse asthma model was observed (FIG. 2). The expression of other members of the calcium activated chloride channel family was determined by PCR (Table 1, Table 2 and FIG. 2). We have identified a murine homolog of CaCC3 (EST AA726662) and we show that the expression is strongly up-regulated (>16 fold) in DRG of the mouse asthma model compared to healthy mice (FIG. 2). In contrast, the expression of the murine homolog (m_CaCC or m_CLCA1) of human CLCA3 was strongly down-regulated in DRG from the mouse asthma model (FIG. 2).

Example 6: By way of example, one novel therapeutic target protein for the treatment of immune and/or inflammatory responses

[00102] LR8/CLAST1 belonging to the family of the tetraspanin (4TM) superfamily has been discovered in a subpopulation of human lung fibroblasts. LR8 mRNA was not detectable by PCR in human smooth muscle cells, endothelial cells or epithelial cells. A murine homolog of LR8 (Signature sequence R1-OS-B1-D3) showed gene (i.e., mRNA) expression in lymph nodes from mice and a down-regulation in the mouse asthma model. Bio-informatics analysis of the LR8 protein confirmed the presumed 4TM structure of the protein and revealed a striking homology with the beta chain of the high affinity IgE receptor (FcεRI) (FIG. 3).

Example 7: Expression of genes in a second mouse model of allergic asthma

[00103] In order to validate the differentially expressed genes, a second, independent mouse model of allergic asthma was used. In this model, Balb/c mice are sensitized by two intraperitoneal injections of ovalbumin (OVA, 10 μg in 2.25 mg Alum adjuvant on days 0 and 7. Subsequently, the mice are exposed to three challenges (days 21, 24, 27) by inhalation of OVA (10 mg/ml) aerosol during 20 minutes. This model is characterized by high serum levels of OVA-specific IgE, strong airway eosinophilia, airway hyperresponsiveness to methacholine and goblet cell hyperplasia, concomitant with the appearance of Th2-like cells in lung tissue. Control-sensitized mice are challenged by inhalation of saline and do not develop airway manifestations of asthma as described above. OVA-sensitized Balb/c mice were challenged by inhalation of either saline or OVA aerosol and at 24 hours after the last challenge, we have isolated the lung, trachea, lung draining (thoracic) lymph nodes (TLN) and dorsal root ganglia (DRG) from these mice. Tissues were immediately stored in RNAlater (Ambion) and within one month transferred to Trizol (GibcoBRL) and total RNA was isolated according to the manufacturer's instructions. ds-cDNA was generated using the SMART-PCR cDNA synthesis kit (Clontech). DNA concentrations were determined spectrophotometrically. Subsequently, these cDNAs were serially two-fold diluted in the wells of 96-well microtiter plates, concentrations ranging from 1.5 ng/μl in sample 1, 0.75 ng/μl in sample 2, down to 0.73 pg/μl in sample 12 (2048x dilution of sample 1).

[00104] Five μl of each sample of each dilution series was used as input in a 20 μl PCR in the following buffer: 66.0 mM Tris-HCl (pH 8.8 at 25°C); 4.0 mM MgCl2; 16.0 mM (NH4)2SO4; 33.2 μg/ml BSA; 340 μM of dGTP, dATP, dTTP and dCTP; and 0.02 Units/μl Taq polymerase (Gibco-BRL).

[00105] In Table 10, a list of specific primer pairs for the indicated genes is given. Two or three sets of primers were combined in each PCR-reaction: one of the two HPRT-primer-pairs and one or two gene-specific primer pairs. Each combination was chosen in such a way that fragments of clearly different lengths were obtained for each gene/EST or for the HPRT-control. Also, primers were cross-checked in such a way that formation of primer-dimers was prevented (i.e., primer pairs with more than 4 bp of complementary sequences -especially when they were present at the end of a primer - were not used together in a PCR-reaction).

[00106] Primer concentrations in the PCR-reactions were 0.5 μM for the gene/EST-specific primers. For the HPRT-primers, the concentrations used ranged from 0.3 μM down to 0.16 μM.

[00107] PCR was performed on a PCT100 (MJ research) or a PE9700 thermal cycler (Perkin Elmer), both with a heated lid (no oil used). A denaturation step of three minutes at 95°C was followed by 33-35 cycles of 30 seconds at 95°C, 40 seconds at 55°C or 68°C (depending on primer sets used) and two minutes at 72°C and then by a final three minutes at 72°C.

[00108] After PCR, 5 μl loading dye was added to each sample and all of the samples were loaded onto 200 ml 2.5% Seakem LE-agarose-gels in 0.5x TBE in 50-well OwL electrophoresis trays and run at 80-100 Volt until the DNAs had migrated long enough to see each gene/EST-specific band (usually 1-2 hours).

[00109] Each gel was photographed with a CCD-camera. At least three photographs were taken from each gel at different diaphragm-settings. All pictures were stored electronically.

[00110] cDNA dilutions from similar tissues obtained from differently treated mice (saline- vs. OVA-challenge) were loaded in such a way in the microtiter plates used to setup the PCR reactions that they would end up next to each other on the gel.

[00111] During the whole procedure described above, multichannel pipets were used to setup the PCR-reactions and to load the gels. Furthermore, master-mixes containing everything but the cDNAs (i.e., including the PCR-buffer, nucleotides, primers and Taq-polymerase) were prepared for each set of primer pairs used.

[00112] In this way experimental variation is kept to a minimum. Also, one can be sure that the total absence of one specific band in one dilution-series is not an artifact if this band is present in another series setup with the same master-mix.

[00113] To determine the level of differential expression, the patterns obtained on gel were scored by eye.

[00114] For the gene/EST-specific bands and for the HPRT control band, the highest dilution in which the band was still present was scored.

[00115] Using the HPRT band as a reference, the difference in gene-expression was scored as a "+1," "+2," "+3," etc., indicating that the gene/EST tested was over expressed at a

respectively two-fold, four-fold, eight-fold, etc., higher level in the OVA than in the saline sample, or as "-1," "-2," "-3," etc., indicating that the gene/EST tested was over-expressed at a, respectively, two-fold, four-fold, eight-fold, etc., lower level in the OVA than in the saline samples.

[00116] As an example in FIG. 1A, the results are shown for the genes with signature sequences OS-B1-C3 and OtS2-C5. The interpretation, based on careful visual inspection (if necessary, using photos taken at different diaphragm-settings) is given by the bars below the photograph: For both saline and OVA the HPRT band is visible down to dilution number 12.

[00117] For OS-B1-C3, no band is visible in the saline-dilution-series, whereas the band can be seen down to dilution number 10 in the OVA-dilution series, indicating that the gene from which this EST is derived is expressed in the Dorsal Root Ganglia of OVA-challenged animals at least 1024-fold (2 to the power 10, in Table 8, this is scored as a 10) more strongly than in saline-challenged mice.

[00118] For OtS2-C5, the band is visible in the first two dilutions in the saline-series and in the first three dilutions in the OVA-series, indicating that the gene from which this EST is derived is two-fold (2 to the power 1, in Table 5, this is scored as a 1) higher expressed after OVA challenge compared to saline challenge.

[00119] In a similar manner in FIG. 1B the results for Cyp2f2 (signature sequence R1-OS-B1-A1) and Gob5 (signature sequence R1-SO-R1-C11) show that Cyp2f2 is highly, but not differentially expressed (scored as a 0 in Table 5), whereas Gob5 is expressed after OVA challenge at least 4096-fold stronger than after saline challenge (2 to the power 12, scored as a 12 in Table 5).

Example 8: Expression of genes in prototypic cell lines

[00120] Allergic asthma is a complex chronic inflammatory disease that involves the activation of many inflammatory and structural cells, all of which participate in the typical pathophysiological changes of asthma (Barnes, 1998 #6873). Many inflammatory cells are recruited to asthmatic airways or are activated in situ. These include mast cells, macrophages, eosinophils, T-lymphocytes, B-lymphocytes, dendritic cells, basophils, neutrophils and platelets. It is now increasingly recognized that structural cells may also be important sources of mediators

in asthma. Airway epithelial cells, smooth muscle cells, endothelial cells and fibroblasts are all capable of synthesizing and releasing inflammatory mediators. Moreover, these cells may become major sources of inflammatory mediators in the airway and this may explain how asthmatic inflammation persists even in the absence of activating stimuli. We have analyzed the expression of many of the identified genes in relevant murine cell types (Table 6). A cell line expressing the relevant gene and the encoding protein can be used for functional studies into the role of the gene/protein and can be used for the screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein. Cell lines were cultured according to guidelines from the "American Type Culture Collection" (www.atcc.org) or as described in literature. The primary dendritic cells were generated from bone marrow cells cultured in the presence of interleukin-4 and granulocyte-macrophage colony-stimulating factor as described in literature (Masurier, 1999 #6874). After culture, cells were harvested and total RNA was extracted using Trizol according to the manufacturer's instructions. 1 μg of total RNA was transcribed into cDNA in a volume of 20 µl. cDNA was used in PCR reactions using gene-specific primer pairs (see Table 4) with a denaturation step of 20 minutes at 95°C, followed by 35 cycles at 94°C for 20 seconds; 55°C for 30 seconds; and 72°C for 30 seconds and then by a final two minutes at 72°C. In some experiments, the cells were activated by a well-known stimulus for that cell type (see Table 6).

[00121] In Table 6, the expression (+) or absence (-) of expression of a particular gene in the respective cell line is shown.

[00122] The mouse calcium-activated chloride channels gob-5 and the murine homolog (EST AA726662) of human CLCA2 are expressed in a prototypic B-lymphocyte cell line (A20). This cell line and other B-lymphocyte cell lines or primary B-cell cultures can be used to determine one or more functions of these ion channels in these cells. Chloride channels are important for cell activation and adhesion. Blockade of one or both of the chloride channels can be used in B-lymphocyte-mediated diseases such as auto-immunity, allograft transplant rejection, allergy and asthma (type I hypersensitivity) and type III hypersensitivity (Arthus reaction, Farmer's lung) in which the disease is at least partially dependent on antibody production such as auto-antibodies, antibodies to graft tissue or antibodies to allergens.

[00123] On the other hand, activation of one or both of these chloride channels can be used in infectious diseases or in combination with vaccines (to protect against infections (viruses, bacteria, fungi, or protozoa) to boost the protective B-lymphocyte-mediated antibody response.

[00124] The mouse calcium-activated chloride channels gob-5 (human CLCA1 homolog) and the murine homolog (EST AA726662) of human CLCA2 and the murine homolog (EST W41083) of human CLCA4 are expressed in prototypic monocyte/macrophage cell lines (J774A.1 and RAW264.7) either under baseline conditions (EST W41083) or upon activation. These cell lines and other macrophage/monocyte cell lines or primary macrophage/monocyte cell cultures can be used to determine one or more functions of these ion channels in these cells. Chloride channels are involved in cell activation and adhesion. Macrophages/monocytes are important effector cells in both the innate and adaptive immune response. Macrophages/monocytes can take up antigens and present these after processing to T-lymphocytes. Macrophages/monocytes can also deliver co-stimulatory signals (B7 family members, CD40, cytokines) to lead to optimal T-cell activation. In particular the production of interleukin-12 by macrophages is important to direct T-lymphocyte responses into the type 1 direction. Type 1 T-lymphocytes are characterized by a particular set of cytokines including interferon-γ. Modulation of one or more of these chloride channels can be used to inhibit or stimulate particular monocyte/macrophage functions such as expression of co-stimulatory molecules (CD40, B7 members) or to inhibit or stimulate the production of cytokines such as interleukin-12 and -18. In this way, inhibition of macrophage function is beneficial in the treatment of Th1-mediated diseases such as auto-immunity and Crohn's disease. Vice versa, stimulation of macrophage function by modulation of these chloride channels is beneficial in the treatment of Th2-mediated diseases such as allergy, asthma, certain types of auto-immunity and ulcerative colitis or in the potentiation of vaccination strategies. Macrophages/monocytes are also an important source of inflammatory mediators such as oxygen radicals, nitric oxide and tumor-necrosis factor-α that play a role in immune responses. Modulation of chloride channels is effective in the limitation of the production and release of these mediators.

[00125] The selective expression of the gene with signature sequence SvO2-1-D10 in the prototypic mast-cell line (P815), the prototypic B-lymphocyte cell line (A20) and the prototypic macrophage/monocyte cell lines (J774A.1 and RAW264.7) demonstrates a potential role of this

gene and the encoding protein in the cellular function of these cell types. Modulation of the expression or activity of this gene/protein is useful in diseases mediated by mast cells (allergy, asthma, multiple sclerosis, etc.), mediated by B-lymphocytes (auto-immunity, allergy, asthma, etc.) or modulated by macrophages/monocytes. These cell lines or other cell lines representing the same cell type or primary cell cultures can be used to determine gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00126] The selective expression of the gene with signature sequence OtS2-G2 in the mast cell line CFTL12 and the primary dendritic cells as well as in the activated mast-cell line P815 and in the activated T-cell line EL4 demonstrates a potential role of this gene and the encoding protein in the cellular function of these cell types. Modulation of the expression or activity of this gene/protein is useful in diseases mediated by mast cells, T-lymphocytes or initiated by dendritic cells. These cell lines or other cell lines representing the same cell type or primary cell cultures can be used to determine gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00127] The selective expression of the gene with signature sequence R1-OS-B1-A3 in the prototypic mast cell line P815 and in the activated prototypic B-lymphocyte cell line A20 demonstrates a potential role of this gene and the encoding protein in the cellular function of these cell types. Modulation of the expression or activity of this gene/protein is useful in diseases mediated by mast cells or B-lymphocytes. These cell lines or other cell lines representing the same cell type or primary cell cultures can be used to determine gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00128] The selective expression of the gene with signature sequence R1-OS-B1-A5 in the prototypic mast cell line P815, the T-cell line EL4 and the prototypic macrophage/monocyte cell line RAW264.7 demonstrates a potential role of this gene and the encoding protein in the cellular function of these cell types. Modulation of the expression or activity of this gene/protein is useful in diseases mediated by mast cells, T-lymphocytes or macrophages/monocytes. These cell lines or other cell lines representing the same cell type or primary cell cultures can be used to

determine gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00129] The selective expression of the gene with signature sequence OtS2-B9 in the T-cell line EL4, in the prototypic macrophage/monocyte cell line J774A.1 and in primary dendritic cells demonstrates a potential role of this gene and the encoding protein in the cellular function of these cell types. Modulation of the expression or activity of this gene/protein is useful in diseases mediated by T-lymphocytes or by macrophages/monocytes or initiated by dendritic cells. These cell lines or other cell lines representing the same cell type or primary cell cultures can be used to determine gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00130] The selective expression of the murine homolog (mCaCC, GenBank Acc. AF052746) of human CLCA3 in the prototypic lung type-II epithelial cell line C10 demonstrates a potential role of this gene and the encoding protein in the cellular function of this cell type. This cell line or other cell lines representing type-II epithelial cells such as the human A549 cell line or primary cell cultures of this cell type can be used to determine the gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein. Type II lung alveolar cells produce surfactant. A deficiency in alveolar surfactant causes respiratory distress syndrome (RDS). Modulation of the expression or activity of this gene/protein is useful in diseases mediated by type-II alveolar cells such as RDS.

[00131] The selective expression of murine DC-SIGN (signature sequence OtS1-B7) in the primary cultures of bone marrow-derived dendritic cells demonstrates a potential role of this gene and the encoding protein in the cellular function of dendritic cells. Bone marrow-derived dendritic cells or cell lines representing dendritic cells such as XS52 cell line or other primary cell cultures of this cell type can be used to determine the gene/protein function and screening of a compound (agonist or antagonist) that modulates at least one of the functions of the gene/protein. Dendritic cells are so-called professional antigen-presenting cells (APC) and thus play a crucial role in the initiation and progression of immune and inflammatory responses mediated by T-lymphocytes. Blockade of mDC-SIGN is beneficial in the treatment of T-lymphocyte-mediated diseases such as allergy, asthma, COPD, auto-immune diseases, inflammatory bowel diseases, allograft rejection and infectious diseases.

Example 9: Identification of full-length sequence of OtS1-B7

[00132] Steps in the identification of the OtS1-B7 as the murine homolog of human DC-SIGN

[00133] 1. The identified cDNA fragment with signature sequence OtS1-B7 was used for BLAST analysis leading to two hits with mouse genomic sequences: GenBank acc. AC73804 and AC3706.

[00134] 2. Gene prediction using GenScan (http://bioweb.pasteur.fr/seqanal/interfaces/genscan.html), BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and ClustalW (http://www2.ebi.ac.uk/clustalw/) led to the construction of a 19619 bp long uninterrupted mouse genomic sequence, designated Contig1A. Contig1A consists of the following overlapping contigs present in AC073804 and AC73706:

nt 1-11054	=	nt 294022-305082 from AC073804
nt 11009-19619	=	nt 237022-228395 from AC073804 (reverse complement)
nt 1805-7790	=	nt 39946-34025 from AC073706 (reverse complement)
nt 6918-15759	=	nt 32026-23233 from AC073706 (reverse complement)

[00135] 3. From contig1A, a gene comprising OtS1-B7, was derived. The characterization of this gene was based on in-silico bioinformatics analysis in combination with "wet" work in the laboratory as described below:

[00136] 4. Gene-prediction combined with extensive BLAST-searches and multiple alignment analyses yielded a putative gene consisting of 10 exons and encoding an mRNA with a length of approximately 1200 bp (Table 7 and FIG. 6).

[00137] 5. Subsequently, primers were developed (Table 8) and used for PCR analysis of the OtS1-B7 gene from cDNA of thoracic lymph nodes obtained from OVA-challenged mice. All primer pairs used yielded fragments after PCR with the lengths predicted by the OtS1-B7-sequence. Sequencing of a set of these overlapping fragments, confirmed that the OtS1-B7 gene-sequence was predicted correctly: no differences with respect to the deduced sequence were found.

[00138] 6. The OtS1-B7 gene comprises the OtS1-B7 fragment:

nt 8426-8463	identical to nt 1-38 of OtS1-B7 (3′-part of exon 7)
nt 8955-9106	identical to nt 39-190 of OtS1-B7 (exon 8)
nt 10386-10495	identical to nt 191-300 of OtS1-B7 (exon 9)
nt 11618-11732	identical to nt 301-415 of OtS1-B7 (5′-part of exon 10)

[00139] 7. In order to obtain the 5′- and the 3′-end of the OtS1-B7 cDNA, a variant of the RACE (rapid amplification of cDNA-ends) was used. At the 5′-end the sequence was shown to have a 5′-UTR of 22 bp. Determination of the 3′-end revealed that apart from the predominant 1.2 kb transcript, an approximately 800 bp longer transcript was present. Both transcripts encode the same 325 bp ORF.

[00140] 8. Based on (i) the strong homology (approximately 50%, see multiple sequence alignment, FIG. 10) of OtS1-B7-ORF with human DC-SIGN, and (ii) the selective expression of OtS1-B7 in the primary dendritic cells (see Example 8) and (iii) the staining of spleen dendritic cells with antibodies to peptides derived from OtS1-B7-ORF (see Example 7), we conclude that we have identified the murine homolog of human DC-SIGN, a Dendritic Cell-specific ICAM-3 Grabbing Nonintegrin.

[00141] 9. The Genetic localization of OtS1-B7 was done by ePCR of the 19619 bp long Contig1A-sequence (http://www.ncbi.nlm.nih.gov/genome/sts/epcr.cgi), which resulted in the identification of marker 440942 (GenBank acc. AI480608). This marker has been mapped to mouse chr 8 (WI-RH Map 13431.25 cR3000), in a chromosomal region syntenic with human 19p13.3, the chromosomal region where DC-SIGN has been located.

[00142] 10. In the 19619 bp Contig1A contig one other gene was predicted by GenScan:

14290-14329	promotor
17688-17690	ATG-start codon
17688-18494	809 bp single exon, BLAST-searches with this exon show that it encodes a retrotransposon with approximately 3000 active copies in the mouse genome
18618-18623	poly-Adenylation-site

[00143] 11. Southern-hybridization of a number of restriction digests (see FIG. 7) of BALB/c genomic DNA was performed using a 1101 bp long Contig1A cDNA fragment

comprising the whole coding region of OtS1-B7 plus 123 bp of the 3'-UTR. This probe was generated by PCR with primers 47 (nt 3655-3684 in Contig1A) and 51 (nt 11861-11891 in Contig1A) and 25 ng of it was radiolabeled with 50 uCi of 32P-labeled alpha-dATP using a Amersham multiprime labelingkit (RPN1600Z, AP Biotech) and then hybridized to alkali blotted BALB/c-restriction digests in Church hybridization buffer at 65°C for about 18 hours, washed two times with 2x SSC/0.1% SDS at RT for a few minutes each, and two times in 2xSSC/0.1% SDS for ten and 90 minutes, respectively, and autoradiographed at -70°C using intensifying screens for two and five days, respectively.

[00144] 12. Results and the interpretation of the Southern-hybridization are shown in FIGS. 7 and 4. FIG. 7 shows the predicted restriction enzyme map for the OtS1-B7-gene. FIG. 8 shows the EtBr-staining of the restriction-digests used (panel A), the autoradiograph after two and five days' exposure time (panels B and C) and the interpretation (panels D and E). Panel D shows a graphical representation of all the hybridizing bands, the thickness of the bands indicates their relative strengths as judged by eye using both exposures. Panel E shows the expected hybridization pattern based on the predicted restriction enzyme map (shown in FIG. 7). The thickness of the bands is drawn proportional to the length of the hybridizing region present in each restriction fragment.

[00145] 13. We conclude that all bands derived from OtS1-B7 which are expected to hybridize with the probe used are indeed present, confirming the correctness of the structure of the predicted gene.

[00146] 14. Also, for all six restriction enzymes used additional hybridizing bands can be observed. In all cases, these additional bands hybridize much more weakly. Therefore, we conclude that in addition to OtS1-B7 a second gene is present in the genome of BALB/c which shares homology to OtS1-B7. Because the lengths of the hybridizing bands for this second gene are different for all six enzymes used and because these bands hybridize much more weakly when compared to the hybridizing OtS1-B7 bands, we conclude that this second gene is distantly related or that it might be a pseudogene.

[00147] 15. For OtS1-B7 itself we conclude that it is present as a single copy per haploid genome in the mouse.

Example 10A: Polyclonal antibodies and immunohistochemistry

[00148] Polyclonal antibodies were prepared to mDC-SIGN (signature sequence OtS1-B7) by immunizing rabbits with immunogenic peptides selected from the mDC-SIGN protein sequence. The peptides used for the immunizations were selected on the basis of extracellular localization and immunogenicity (Eurogentec, Belgium). KLH conjugated peptides used for antibody production:

AA 77-92 + C (SEQ ID NO: 6): H2N - KTP NTE RQK EQE KIL QC - CONH2 (17 AA) and

AA 275-289 + C (SEQ ID NO: 7): H2N - SRF QKY WNR GEP NNI C - CONH2 (16 AA)

[00149] Peptides were synthesized and polyclonal antibodies were generated by Eurogentec according to their standard procedures. In short, peptides were synthesized by Fmoc chemistry and coupled to Keyhole Limpet Hemocyanin (KLH). Both KLH coupled peptides were mixed and used to immunize (200 μg in Freund's adjuvant) two rabbits on days 0, 14, 28 and 56. Serum was obtained prior to immunization (pre-serum, day 0) and at 35, 66 and 87 days after immunization (immune serum).

[00150] Both rabbits generated antibodies to either of the peptides as demonstrated by an ELISA using the peptide as coat. The polyclonal antibodies were used for immunohistochemistry. Cryostat sections (5 μm) of trachea, thoracic lymph nodes, spleen and dorsal root ganglia were used for immunohistochemistry. After blocking by incubation with 10% normal goat serum, tissues were washed and incubated with different dilutions (1:1000 to 1:5000) of either pre-immune- or immune-serum (day 87). Thereafter, tissues were incubated with anti-rabbit immunoglobulin antibody (DAKO) and after washing tissues were incubated with substrate DAB (Sigma) according to the manufacturer's instructions. After fixation and counter staining with hematoxyline, tissues were analyzed by light microscopy.

[00151] In the spleen from naive mice, there is a strong and localized staining of marginal zone dendritic cells for mDC-SIGN in the tissues incubated with immune serum compared to pre-immune serum FIG. 8). Thus, there is expression of mDC-SIGN protein in spleen marginal zone dendritic cells. This strongly confirms that we have identified the murine homolog of human DC-SIGN.

[00152] In dorsal root ganglia from saline-challenged control mice, there is a very weak staining for mDC-SIGN in the tissues incubated with immune serum versus pre-immune serum (FIG. 9). In the dorsal root ganglia from OVA-challenged mice (as described in Example 7), there is a very strong staining for mDC-SIGN in the tissues incubated with immune serum compared to pre-immune serum. Thus, there is a weak expression of mDC-SIGN protein in DRG from control mice and a very strong expression of mDC-SIGN protein in DRG from OVA-challenged mice.

[00153] In the trachea from saline-challenged control mice, there is staining of epithelial cells for mDC-SIGN in the tissues incubated with immune serum versus pre-immune serum. In the trachea from OVA-challenged mice (as described in Example 7), there is a strong staining of epithelial cells for mDC-SIGN in the tissues incubated with immune serum compared to pre-immune serum (FIG. 10). Thus, there is expression of mDC-SIGN protein in tracheal epithelial cells from control mice and a stronger expression of mDC-SIGN protein in DRG from OVA-challenged mice.

[00154] In the thoracic lymph nodes (TLN) from saline-challenged control mice, there is staining of dendritic cells for mDC-SIGN in the tissues incubated with immune serum versus pre-immune serum (FIG. 11). In the TLN from OVA-challenged mice (as described in Example 7), there is a strong staining of dendritic cells for mDC-SIGN in the tissues incubated with immune serum compared to pre-immune serum. Thus, there is expression of mDC-SIGN protein in TLN from control mice and a stronger expression of mDC-SIGN protein in TLN from OVA-challenged mice.

[00155] Antibodies (mono- or polyclonal or fragments thereof) to DC-SIGN can be used for the isolation, staining (immunohistochemistry, flow cytometry) and functional studies using murine dendritic cells.

Example 10B: OtS1-B7 blockage by monoclonal antibody ERTR9 attenuates allergen-induced airway manifestations of asthma

[00156] We examined whether treatment with ERTR9, a specific monoclonal antibody that binds to OtS1-B7 (Geitenbeek et al., 2002; Kang et al., 2003), was able to affect the induction of airway manifestations of asthma in a mouse model of allergic asthma. Balb/c mice

were divided into two groups of twelve animals (group I and group II) including six treatment and six control animals. Treatment group I received an intraperitoneal injection of ERTR9 (2mg) one hour prior to sensitization and one hour prior to the first of three OVA inhalation challenges. Treatment group II received an intraperitoneal injection of ERTR9 (1 mg) one hour prior to the first of three OVA inhalation challenges only. Control group I received 2 mg of rat IgM one hour prior to sensitization and one hour prior to the first of three OVA inhalation challenges, and control group II received 1 mg of rat IgM one hour prior to the first of three OVA inhalation challenges only. The mice were sensitized with OVA in alum adjuvant one hour after treatment with antibodies and again after seven days, as described previously (Deurloo et al., 2001). Blood samples were obtained twenty days after treatment to determine OVA-specific serum IgE antibody levels. Subsequently, the airway responsiveness to the bronchospasmogenic stimulus methacholine was determined by whole body plethysmography as described earlier (de Bie et al., 2000). Starting on day 24, the mice were challenged three times, once every third day, by inhalation of OVA (2mg/ml). Airway responsiveness to methacholine was determined 24 hours after the last inhalation challenge, on day 31. The lungs were lavaged to determine the numbers of infiltrating inflammatory cells in the lung lumen and blood samples were obtained to determine OVA-specific serum IgE antibody levels.

[00157] Airway responsiveness to methacholine was significantly increased ($P<0.05$) after OVA challenge as compared to prechallenge values in mice treated with control antibody (FIGS. 18A and 18B). In group I, treatment with ERTR9 before sensitization and before challenge, attenuated the allergen-induced airway hyperresponsiveness to methacholine (FIG. 18A). In group II, mice treated with ERTR9 prior to challenge only, airway responsiveness to methacholine was also significantly increased after OVA challenge as compared to pre-challenge values ($P<0.05$). However, airway responsiveness in ERTR9-treated mice was significantly lower than in control mice ($P<0.05$) (FIG. 18B).

[00158] Serum levels of OVA-specific IgE were increased after OVA challenge as compared to pre-challenge values (Tables 9A and 9B). Interestingly, treatment with ERTR9 prior to sensitization and challenge inhibited the up-regulation of serum OVA-specific IgE levels after OVA challenge in group I (Table 9A). In group II serum IgE levels were increased in the

treatment and control groups, however, serum IgE levels are reduced by almost 50% in the ERTR9-treated mice.

[00159] The number of infiltrating eosinophils in the BALF of mice treated with ERTR9 before sensitization and challenge (group I) was significantly reduced compared to control mice (FIG. 19A). In mice treated with ERTR9 before challenge only (group II), the number of infiltrating eosinophils in the BALF was reduced by almost 50% (FIG. 19B).

[00160] It is concluded that the blockade of OtS1-B7 binding sites, by treatment with the monoclonal antibody ERTR9, attenuates allergen-induced airway manifestations of asthma. These data show that OtS1-B7 plays an important role in the initiation and progression of the OVA-induced immune- and inflammatory response.

Example 11: Generation of mDC-SIGN "knock-out" mouse

[00161] In order to study the role of mDC-SIGN (signature sequence OtS1-B7), a targeting construct was designed to knock-out this gene in mice.

[00162] The targeting-construct contains a left arm encompassing part of intron 4, exon 5, intron 5 and part of intron 6, followed by a PGK-hyg cassette and a left arm encompassing part of intron 8, exon 9, intron 9, exon 10 and several kb of the region downstream of OtS1-B7 (see FIG. 12).

[00163] After electroporation of the construct into 129/OLA E14 ES-cells, hygromycin resistant clones will be screened by Southern analysis or LD-PCR to obtain clones which correctly targeted and which do not contain random insertions of the targeting construct (diagnostic restriction fragments and the hybridization probe to be used are indicated in the figure).

[00164] Some of these clones will be used for blastocyst-injections after which they will be transferred to 129 mice to generate mDC-SIGN knock-out mice.

Further examples of use:

[00165] DC-SIGN can be blocked by mono- and polyclonal antibodies or fragments thereof directed against DC-SIGN (protein or peptide fragments); by the soluble protein ligands ICAM-2 and -3 or fragments thereof; by HIV gp120 or fragments thereof; by mannose

carbohydrates such as mannan and D-mannose; fucose carbohydrates such as L-fucose; plant lectins such as concanavalin A; antibiotics such as pradimicin; sugars such as N-acetyl-D-glucosamine and galactose; and the $Man_9GlcNAc_2$ oligosaccharide of soybean agglutinin. Calcium-activated chloride channels (CLCA1-4) can be blocked by mono- and polyclonal antibodies or fragments thereof directed against the ion channel (protein or peptide fragments); known nonspecific chloride channel antagonists such as 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS), 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPD), niflumic acid, and the anti-allergic drug cromolyn; Ion-channel toxins such as FTX-3.3 or synthetic analogues such as sFTX-3.3 and argiotoxin. Antibodies (mono- or polyclonal or fragments thereof) to murine DC-SIGN can be used for (i) staining of dendritic cells by immunohistochemistry, flow cytometry, etc.; (ii) for isolating and/or purifying dendritic cells from a biological sample or a culture medium; (iii) functional studies into the role of DC-SIGN.

[00166] A number of genes are strongly increased in expression in DRGs obtained from "asthmatic," OVA-challenged mice compared to control, saline-challenged mice such as genes with signature sequence: SvO2-1-A11; SvO2-1-C8, R1-OS-B1-C3; OtS2-B9, R1-OS-B1-D6, SvO2-1-B7 (with KPI domain) and OtS1-B7. Blockade of one or more of these genes or the encoding proteins by selective antagonists inhibits the excitability of sensory neurons and thereby prevents or decreases (1) the neurogenic component of inflammatory diseases, (2) hyperalgesia during inflammatory responses and (3) cough due to airway inflammation.

[00167] The calcium-activated chloride channels Gob-5 and the murine homolog (EST AA726662) of human CLCA2 as well as the gene OtS2-C3 (signature sequence ID) are up-regulated in trachea derived from "asthmatic" mice compared to "healthy" control mice. Blockade of one or more of these genes or the encoding proteins is beneficial in the treatment of allergic airway diseases.

[00168] The murine homolog (EST AA726662) of human CLCA2 (also called CaCC3) is strongly up-regulated in DRGs obtained from "asthmatic" mice compared to "healthy" control mice. This corroborates the data presented here in Example 5. Dorsal root ganglia contain sensory nerve bodies that are involved in neurogenic inflammation which contributes to allergic

inflammation and pain (inflammatory hyperalgesia). Interference with human calcium-activated chloride channel CLCA2 may limit neurogenic inflammation in asthma and other diseases with a neurogenic inflammatory component. Furthermore, cough, which is a prominent symptom of asthma, is believed to be a result of sensory nerve activation.

[00169] Blockade of hCLCA2 (or the murine homolog) by selective antagonists inhibits the excitability of sensory neurons and thereby prevents or decreases (1) the neurogenic component of inflammatory responses, (2) hyperalgesia during inflammatory responses and (3) cough due to airway inflammation.

Role of DC-SIGN:

[00170] Immature dendritic cells (DCs) migrate from the blood into peripheral tissues where they capture and process antigens and subsequently migrate to lymphoid organs to either activate or tolerize T-lymphocytes in an antigen-specific way. DCs play an important role in allergic sensitization as well as in the induction of antigen-induced airway manifestations of asthma. In agreement herewith, we recently showed that passive transfer of ovalbumin (OVA) primed spleen-derived DCs strongly potentiates the development of allergic airway inflammation, airway hyperreactivity and Th2-associated cytokine production upon subsequent antigen inhalation. These data clearly demonstrate that DCs are key regulatory cells in the initiation and progression of Th2-dominated allergic airway responses. Recently, a DC-specific receptor called DC-SIGN (DC-Specific ICAM-3 Grabbing Nonintegrin) was identified. DC-SIGN is a mannose binding cell surface receptor member of the C-type lectin family and appears to be expressed exclusively by DCs. DC-SIGN mediates the interaction between DCs and resting T-cells via ICAM-3 and has recently been shown to be important in DC-induced proliferation of human resting T-cells *in vitro*. Moreover, DC-SIGN has also been shown to be involved in trans-endothelial migration of DCs via interaction with ICAM-2 on human vascular endothelial cells. These data suggest an important role for DC-SIGN in the trafficking of DCs. We have discovered the murine homolog of DC-SIGN by differential gene expression using lung-draining lymph nodes obtained from control and OVA-challenged mice. The full-length sequence of murine DC-SIGN shows strong (50%) homology to human DC-SIGN. Rabbit polyclonal antibodies to murine DC-SIGN-derived peptides were generated and used for

immunohistochemistry. The staining of spleen and lymphoid tissues from mice using this antibody demonstrate that murine DC-SIGN is expressed on DCs.

[00171] Another important function of DC-SIGN in the initial dissemination of HIV-1 shortly after infection. DC-SIGN is highly expressed on DC present in mucosal tissues and binds to the HIV-1 envelope glycoprotein gp120. DC-SIGN does not enable viral entry into DC, but protects the virus until DC migrate to T-cell-rich lymph nodes. Here, DC-SIGN promotes efficient infection in trans of CD4+ T-cells. Transmission of HIV-1 by DC to T-cells is inhibited by the blocking antibodies against DC-SIGN.

[00172] Interestingly, we demonstrate the expression of DC-SIGN at the protein level using polyclonal antibodies in mouse airway epithelial cells and in sensory neurons present in dorsal root ganglia.

[00173] Epithelial cells in the respiratory system are not passive bystanders during assault of the epithelial barrier but participate actively in the inflammatory response to defend the airway. Because epithelial cells are located at sites of contact with the external environment, they are often the first cells to interact with potential microbial pathogens. Indeed, bacterial adherence to epithelial cells may be a prerequisite for colonization and infection and through this interaction epithelial cells may have the opportunity to detect and respond to pathogens independent of signals from other cell types in the respiratory system. The capacity for epithelial cells to directly detect microbial pathogens and immediately initiate expression of genes directed toward defense may allow for more efficient activation of the inflammatory response. Although several molecules that participate in airway defense have been identified, the activation and coordination of factors that result in a rapid and effective inflammatory response at the epithelial surface are only beginning to be elucidated.

[00174] One mechanism for epithelial cells to participate in airway defense is through coordination of leukocyte influx and activation by expression of adhesive surface proteins and secretion of chemotactic molecules (interleukin-8, eotaxin, RANTES). DC-SIGN can play an important role in both the adhesion of leukocytes expressing ICAM molecules (in particular ICAM-2 and ICAM-3) and in the adhesion of pathogens (bacteria, fungi, parasites and viruses). Antibodies to DC-SIGN or compound that block the interaction between either ICAM molecules

and DC-SIGN or the interaction between sugar moieties or other surface molecules of pathogens and DC-SIGN can be used to prevent or treat infections with these pathogens.

[00175] Blockade of DC-SIGN is useful as a treatment for allergic asthma, COPD or other inflammatory diseases of the airways.

[00176] Dorsal root ganglia contain sensory nerve bodies that are involved in neurogenic inflammation which contributes to allergic inflammation and pain (inflammatory hyperalgesia). Furthermore, there is strong and convincing evidence for interactions between the immune and peripheral nervous systems. Many regulatory molecules are candidate mediators for communication between inflammatory cells and nerves. There is substantial evidence that various immune (lymphocytes) and inflammatory cells (mast cells, eosinophils, etc.) are in close contact with nerves. Lymphoid tissues, mucosal sites (gut, airway) and skin are densely innervated and contacts between nerves and inflammatory cells have been demonstrated. Mast cells are in close proximity to nerves in mucosa and skin and nerve stimulation has been reported to cause mast cell activation. Such data suggest a dynamic interplay between the immune and nervous systems during immune and inflammatory responses. In agreement herewith, receptors for various neurotransmitters, in particular neuropeptides (substance P, CGRP, etc.) are present on all immune- and inflammatory cells. A close contact between these immune- and inflammatory cells and the neurons is required for these neuronal mediators to be effective. DC-SIGN is an important adhesion molecule on sensory nerves that can bind to ICAM molecules (ICAM-2 and -3) on immune and inflammatory cells thereby establishing the close contact required for this neuro-immune interaction. Blockade of DC-SIGN inhibits the neuronal component of immune- and inflammatory responses and is beneficial in inflammatory diseases such as auto-immunity, allergy, asthma, inflammatory bowel disease, etc.

[00177] Neurotropic viruses such as herpes simplex virus (HSV) and human immunodeficiency virus (HIV) can infect peripheral neurons.

[00178] Cell surface expression of DC-SIGN in neurons may be an important step in the infection of neurons with neurotropic viruses such as HSV and HIV mediated by glycosylated viral envelope proteins. Compounds that inhibit the interaction between DC-SIGN and the viral glycoproteins are useful in prevention and treatment of these neurotropic viral infections.

[00179] The selective expression of CLCA2 in the prototypic human epithelial cell line demonstrates a role of this gene and the encoding protein in the cellular function of these cell types. Chloride channels play a role in production and secretion of mucus and chemotactic molecules (interleukin-8, eotaxin, RANTES) by epithelial cells. CLCA2 also can play a role in cellular adhesion. This cell line and other epithelial cell lines can be used to study the role of human CLCA2 gene or the encoding protein in lung epithelial cell function such as mucus production and secretion and can be used for the screening of compounds (agonist or antagonist) that modulates at least one of the functions of the gene/protein.

[00180] Blockade of this ion channel will inhibit mucus production and is, therefore, beneficial in the treatment of airway diseases associated with increased mucus production such as asthma and COPD.

[00181] Compounds that up-regulate the expression of CLCA2 in human epithelial cells are useful in the treatment of patients with cystic fibrosis which have a defect in cAMP-mediated chloride secretion.

[00182] A number of genes are strongly increased in expression in DRGs obtained from "asthmatic," OVA-challenged mice compared to control, saline-challenged, mice such as genes with signature sequence: SvO2-1-A11; SvO2-1-C8, R1-OS-B1-C3; OtS2-B9, R1-OS-B1-D6, SvO2-1-B7 (with KPI domain) and OtS1-B7. Blockade of one or more of these genes or the encoding proteins by selective antagonists inhibits the excitability of sensory neurons and thereby prevents or decreases (1) the neurogenic component of inflammatory diseases, (2) hyperalgesia during inflammatory responses and (3) cough due to airway inflammation.

[00183] The calcium-activated chloride channels Gob-5 and the murine homolog (EST AA726662) of human CLCA2 as well as the gene OtS2-C3 (signature sequence ID) are up-regulated in trachea derived from "asthmatic" mice compared to "healthy" control mice. Blockade of one or more of these genes or the encoding proteins is beneficial in the treatment of allergic airway diseases.

[00184] The murine homolog (EST AA726662) of human CLCA2 (also called CaCC3) is strongly up-regulated in DRGs obtained from "asthmatic" mice compared to "healthy" control mice. Dorsal root ganglia contain sensory nerve bodies that are involved in neurogenic inflammation which contributes to allergic inflammation and pain (inflammatory hyperalgesia).

Interference with human calcium-activated chloride channel CLCA2 may limit neurogenic inflammation in asthma and other diseases with a neurogenic inflammatory component. Furthermore, cough, which is a prominent symptom of asthma, is believed to be a result of sensory nerve activation. Blockade of hCLCA2 (or the murine homolog) by selective antagonists inhibits the excitability of sensory neurons and thereby prevents or decreases (1) the neurogenic component of inflammatory responses, (2) hyperalgesia during inflammatory responses and (3) cough due to airway inflammation. It is demonstrated herein that the human lung epithelial cell line expresses the CLCA2 gene constitutively FIG. 13). The selective expression of CLCA2 in the prototypic human epithelial cell line demonstrates a role of this gene and the encoding protein in the cellular function of these cell types. Chloride channels play a role in production and secretion of mucus and chemotactic molecules (interleukin-8, eotaxin, RANTES) by epithelial cells. CLCA2 also can play a role in cellular adhesion. This cell line and other epithelial cell lines can be used to study the role of human CLCA2 gene or the encoding protein in lung epithelial cell function such as mucus production and secretion and can be used for the screening of compounds (agonist or antagonist) that modulates at least one of the functions of the gene/protein. Blockade of this ion channel will inhibit mucus production and is, therefore, beneficial in the treatment of airway diseases associated with increased mucus production such as asthma and COPD.

[00185] Compounds that up-regulate the expression of CLCA2 in human epithelial cells are useful in the treatment of patients with cystic fibrosis which have a defect in cAMP-mediated chloride secretion.

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TABLES

[00186] Table 1: Identification of differentially expressed genes in "asthmatic" mice compared with "healthy" control animals.

[00187] Array [1]: Expression ratio (asthma:healthy) obtained by hybridization of a cDNA micro-array with fluorescently labeled amplicons (Cy5 versus Cy3) derived from "asthma" and "healthy" mice.

[00188] Blot [2]: Expression ratio (asthma:healthy) obtained by virtual northern blotting of amplicons and hybridization with fluorescently labeled specific, individual gene fragments.

[00189] a) Known genes up-regulated in "asthma" versus "healthy" mice.

Signature Sequence	**Sequence/gene**	**Human homolog**	**Array[1]**	**Blot[2]**
R1-SO-R1-A11	Igγ	IgGγ	2.09	10
StO1-A10	Igε	Igε	2.08	
SvO2-1-C11	Igμ	Igμ		
StO1-A12	IgG1 H chain	IgG1 H chain	2.20	
R1-SO-R1-B7	Igκ	Igκ	2.36	4
R1-SO-R1-A7	SLPI (secretory leukocyte protease inhibitor)	SLPI	3.19	10
R1-SO-R1-E7	Tdt (terminal deoxynucleotidyl transferase)	Tdt	3.65	
StO1-B3	CsA-19	CsA-19	1.57	
StO1-B5	MHC-II (I^{A-d})	MHC-II	3.11	
R1-SO-R1-C11	Gob-5 (ca^{2+} activated Cl^- channel)	CaCC1/CLCA1	1.88	2
R1-SO-R1-E11	Pendulin	Rch1/Srp1α/ Importin-α	0.84	2
R1-SO-R1-A12	EST AA277412; AW910210; AI591665; AA980800	CDC42-GAP (GTPase-activating protein)	1.02	2
StO1-C1	Aspartyl aminopeptidase	Aspartyl aminopeptidase	1.41	
StO1-D3	RA70 (mouse retinoic acid responsive gene)	SKAP-HOM (SKAP55 homolog)	0.77	
SvO2-1-B7	APLP2 (amyloid β precursor-like protein)	APLP2		

Signature Sequence	Sequence/gene	Human homolog	Array[1]	Blot[2]
SvO2-1-D8	GDP-dissociation inhibitor (ly-GDI)	Ly-GDI		
SvO2-1-C4	Plastin-2 (PLS2)	L-Plastin		
SvO2-1-C12	Ubiquitin/60s			
SvO2-1-A4	H2-Oa (MHC-II)	HLA-DNA		
SvO2-1-G3	EST AI327412; AA140026	RNA Polymerase-II subunit (POLR2G)		
SvO2-1-A8	EST AW546508	Phospholipase- C γ2 (PLCγ2)		
SvO2-1-D4	EST AW044803; AA823969; AA869959	Clathrin (CLTCL2)		
SvO2-1-D5	EST BB000142	Glutamyl-propyl-tRNA synthethase (EPRS)		

[00190] b) Expressed sequence tags (ESTs) up-regulated in "asthma" versus "healthy" mice

Signature sequence	Sequence/gene	Human homolog
SvO2-1-D10	EST AI153476; AA537538	
SvO2-1-A11	EST AI451488	AW173082
SvO2-1-C8	EST AA023597; AW476575	
SvO2-1-E6	EST AI587693; AA499481; AU080538	
SvO2-1-F1	EST C77954	

[00191] c) Known genes down-regulated in "asthma" versus "healthy" mice.

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
R1-OS-B1-B1	PIN (protein inhibitor of NnoS)	Dynein light chain	1.44	0.7

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
R1-OS-B1-A1	CYP2F2 (cytochrome P450 naphthalene hydroxylase)	CYP2F1	0.35	0.1
R1-OS-B1-B6	IDH-α (NAD^+-dependent isocitrate dehydrogenase)	NAD^+ dependent isocitrate dehydrogenase	0.71	0.5
R1-OS-B1-G3	Stat-1	Stat-1	0.65	0.3
R1-OS-B1-H1	SEPP1 Selenoprotein P	SEPP1	0.52	0.5
R1-OS-B1-C5	Decorin	Decorin	0.40	0.3
OtS2-F2	Cathepsin B	Cathepsin B	0.56	
OtS2-E6	Gluthation-S-transferase mu 2 (Gstm2)	Gluthation-S-transferase	0.40	
OtS2-H2	Breast heat shock 73 protein (Hsc73)	HSP 70	0.60	
OtS2-B12	Sulphated glycoprotein-2 isoform APOJ/Clu	Clusterin	0.46	
R1-OS-B1-D3	LR8/CLAST1	LR8	0.54	0.5
R1-OS-B1-C1	EST AW211263; AI194829; AI098607; W08910	Mitochondrial trifunctional protein	0.55	0.7
R1-OS-B1-A2	UBP43 (ubiquitin-specific protein)	ISG43	0.80	0.5
R1-OS-B1-D5	Ferritine	Ferritine	0.45	1.0
OtS2-B4	Unidentified mitochondrial gene		0.50	
OtS2-A1	Mitochondrial cyt-C oxidase subunit I		0.43	
OtS2-C10	Mitochondrial enoyl-CoA hydratase (rat)	Mitochondrial enoyl-CoA hydratase	0.34	
OtS2-A6	AOP2 (antioxidant protein 2)	AOP2	0.45	
OtS2-D9	IL-2R-γ	IL-2R-γ	0.51	
OtS2-A7	EST AA475628	TIS11d (early response gene)/tristetraprolin	1.12	
OtS2-C6	HSP (84 kd heat shock protein)	HSP 90	0.75	
OtS2-A10	IFNγR (interferon-γ receptor)	IFNγR	0.32	
OtS2-C11	Ornithine decarboxylase (Odc)	Ornithine decarboxylase	0.55	
OtS1-C11	Stearoyl-CoA desaturase 1 (SCD1)	Stearoyl-CoA desaturase	0.38	
OtS2-B10	MUSLYSM4 (mouse		0.54	

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
	lysozyme gene)			
OtS2-D8	Calnexin	Calnexin	0.61	
R1-OS-B1-D6	Plunc	Plunc	0.39	

[00192] d) Expressed sequence tags (ESTs) down- regulated in "asthma" versus "healthy" mice.

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
OtS2-D3	EST AI451901; AW826053; AA712022, partially similar to mouse CR2		0.74	
OtS2-D2	EST AA423205, similar to X57528 mouse retinoic acid receptor-alpha		0.87	
OtS2-D10	Similar but not identical to mouse CD59 (complement inhibitory protein)		0.53	
OtS1-B7	EST AA543877; AA914211 (similar but not identical to macrophage lectin-2)	Similar but not identical to membrane C-type lectin 2	0.43	
R1-OS-B1-C3	EST AA691014; AW321759		0.84	0.5
OtS2-G2	Mouse JHL1 (AF165227)		0.58	
R1-OS-B1-H6	EST AI450028, AW548213; AA672579	MUM2 (AF129332)	0.83	0.25
R1-OS-B1-A3	EST AA512682; AI314236		0.65	0.7
R1-OS-B1-C4	EST AA396183 (similar to rat ROD1)	ROD1	0.66	0.5
R1-OS-B1-A5	EST AW490156 (similarity to dynein beta subunit)	EST AI358291; AI623698	1.02	0.3
R1-OS-B1-B2	EST AI835555			0.7
OtS2-C1	EST AA939676; AA125221; AA798681; AA869527		0.77	
OtS2-D7	EST AU078971; AA178650;		1.60	

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
	AA231343			
OtS2-B9	EST AA792488; AA177706		0.37	
OtS2-A9	EST AA273304; AA270364; AA671609	AF143676 (multi-spanning nuclear envelope membrane protein)	0.56	
R1-OS-B1-C6	EST AI874718; AA498063; AA615985		0.81	
OtS2-C3	EST AI788596; AI892968; AA939676		0.66	
OtS2-B6	EST AI528153; AA982059; AW488424		0.67	
OtS2-A12	EST AA940560 (Rho-GAP domain)	AF217507	0.65	
OtS2-B3	EST AL022972	AW958031	1.43	
OtS2-A5	EST AA433598; AL118320; AI507121		1.08	
OtS2-C4	EST AW913417; AI647667		0.68	

[00193] e) Genes down-regulated in "asthma" versus "healthy" mice.

Signature sequence	Sequence/gene	Human homolog	Array [1]	Blot [2]
R1-OS-B1-E5	See FIG. 4 for sequence		0.97	0.7
OtS2-C5	See FIG. 4 for sequence		0.35	

[00194] Table 2: members of the calcium-activated chloride channel family.

Human gene/protein	Murine homolog	Signature sequence
CaCC1 / CLCA1	Gob-5	R1-SO-R1-C11
CaCC2	EST W41083	
CaCC3 / CaCL2	EST AA726662	
CLCA3	CaCC / CLCA1	

[00195] **Table 3:** An example of some of the differentially expressed genes involved in the regulation/activation of T-lymphocytes from Table 1.

Up-regulated genes/proteins	**Signature sequence**	**Down-regulated genes/proteins**	**Signature sequence**
CsA-19	St-O1-B3	IL2-R-gamma	OTS2-D9
Pendulin	R1-SO-R1-E11	IFN-γ-R	OTS2-A10
RA70	StO1-D3	Stat-1	R1-OS-B1-G3
Ly-GDI	SV02-1-D8		
Plastin-2	SVO2-1-C4		
EST: RNA Polymerase-II subunit	SVO2-1-G3		
EST: Clathrin	SVO2-1-O4		
EST: Cdc42-GAP	R1-SO-R1-A12		

[00196] Table 4: Primer pairs used for semi-quantitative PCR analysis of indicated (signature sequence) genes and their respective product length.

Signature sequence	**sense primer "forward"**	**anti-sense primer "reverse"**	**product length (base pairs)**
OtS1-B7	ATGAGTGACTCCACAGAAGCCAA GATGCAG (SEQ ID NO: 8)	AAGAACAGGAAGGAGAGCAGCTGCAGG AC (SEQ ID NO: 9)	415
SvO2-1-B7	1: ATACACAGGCTGTTCCCGTT (SEQ ID NO: 10) 2: AAGTGGTGGAAGACCGTGAC (SEQ ID NO: 12)	1: ATGATGAAGCCTCCCGTG (SEQ ID NO: 11) 2: AAATGCTGGATGAGGGTCTG (SEQ ID NO: 13)	568 504 with KPI 336 without KPI
SvO2-1-D8	TGGACCTTACTGGGGATCTC NNN (SEQ ID NO: 14)	ACTCTTCTGGTGGGTGAGGA (SEQ ID NO: 15)	401
OtS2-A6	ATGCCCGGAGGTTTGCTTCT (SEQ ID NO: 16)	TGCCTGTCAGCTGGAGAGAG (SEQ ID NO: 17)	514
R1-OS-B1-D3	AGTCAAAGTGGCCTCCACAC (SEQ ID NO: 18)	CAAGAGCACAGCTCACAAGC (SEQ ID NO: 19)	197
R1-OS-B1-A1	CAGCCATCTTGCTTCTCCTC (SEQ ID NO: 20)	ACAGAGCGGCTCAGGATAAA (SEQ ID NO: 21)	508
R1-SO-R1-C11	GCCTTCGGACAGCATTTACA (SEQ ID NO: 4)	TGCGTTGTCCAGGTGATAAG (SEQ ID NO: 5)	412
EST AA726662	GGTTGAGGAGCGAATGGAAGAGC (SEQ ID NO: 22)	ATTGCCCACGGCGCTATCCA (SEQ ID NO: 23)	362
EST W41083	AGCTAGTCCTTCTGGACAACGGTG C (SEQ ID NO: 24)	TGTTGGATGGTCCCGAACTCAAA (SEQ ID NO: 25)	654
mCaCC	ATTAGTCACATTTGACAGCGCTGC C (SEQ ID NO: 26)	TGGGAGACGCTGCCACTTGTAGAT (SEQ ID NO: 27)	414
SvO2-1-D10	TTTGAACCTCGCCCACTGTG (SEQ	GCACCCATACTGATAGCTCTCA (SEQ ID	806

Signature sequence	sense primer "forward"	anti-sense primer "reverse"	product length (base pairs)
	ID NO: 28)	NO: 29)	
SvO2-1-A11	TCTTCCTTTGCTCAGACACACAGG (SEQ ID NO: 30)	TTCCCCCCTCTTTACTCCTGG (SEQ ID NO: 31)	418
SvO2-1-C8	GAAGACGCCACTGTTCCGAA (SEQ ID NO: 32)	TGAGAGTGGAGGCTGCCGTC (SEQ ID NO: 33)	635
SvO2-1-E6	TCGACCCGAATCTGTTTGCA (SEQ ID NO: 34)	TTTTCCCGCTTCCTGTCTCAG (SEQ ID NO: 35)	633
OtS2-D3	TCAGAAGAAGCTTTGAACTTTGG (SEQ ID NO: 36)	ATCCTGGGGCAGCAAAAA (SEQ ID NO: 37)	264
OtS2-D10	GAAGGTGTCTGTGAAGCCTGTGG (SEQ ID NO: 38)	TGCATTCCGGCTACAGCATAGA (SEQ ID NO: 39)	307
mCD59	CAGTCACTGGCGATCTGAAAAG (SEQ ID NO: 40)	TGCATTCCGGCTACAGCATAGA (SEQ ID NO: 41)	250 370 (5′ UTR variant)
R1-OS-B1-C3	ACAAGGCTTTAAGACTGCGACAGC (SEQ ID NO: 42)	GAGAGCCGGGAGAGTTTGCTAT (SEQ ID NO: 43)	665
OtS2-G2	AGTGCACTTGCATGGAGCTCA (SEQ ID NO: 44)	ACAAGGGGGAGAAGCAGCTG (SEQ ID NO: 45)	428
R1-OS-B1-H6	GAGCTGACCAACATGGGTGC (SEQ ID NO: 46)	GCGGGCACAGAGGATTCTTC (SEQ ID NO: 47)	227
R1-OS-B1-A3	GATCAACGCAAGCTCTTGGC (SEQ ID NO: 48)	CTTTGCCCAAAATAGAGCCA (SEQ ID NO: 49)	210
R1-OS-B1-C4	ACACTGTTGGGGAAAACGAG (SEQ ID NO: 50)	GACTGAAGCAGCTCAAGACC (SEQ ID NO: 51)	121
R1-OS-B1-A5	ACCGAGACCAAGCTGCAGTG (SEQ ID NO: 52)	GGCGAGGCTCCCACTTACTC (SEQ ID NO: 53)	413
R1-OS-B1-B2	CTGAGGGGAGCCTGCTGGAA (SEQ ID NO: 54)	CCCAGTGGATGCCTGAAACA (SEQ ID NO: 55)	271

Signature sequence	sense primer "forward"	anti-sense primer "reverse"	product length (base pairs)
OtS2-C1	CCTAAGCGCTGGGATTTTAC (SEQ ID NO: 56)	TGATCCTCATTGCAGAAGTTTAGCT (SEQ ID NO: 57)	379
OtS2-D7	TTTTTCATGGCTTCCTGCGG (SEQ ID NO: 58)	CACCCCTCTGCGACAAGACA (SEQ ID NO: 59)	403
OtS2-B9	GACCTGGACGAGACCCTGGT (SEQ ID NO: 60)	AGAAAATTCAGCCACTGCCA (SEQ ID NO: 61)	150
OtS2-A9	TCAGGAACTGAGTTCTCCAG (SEQ ID NO: 62)	CTGGCTCTTCTCTTTACCCT (SEQ ID NO: 63)	280
R1-OS-B1-C6	CATCAGAGCCAGCTATGCCG (SEQ ID NO: 64)	GGAAGCATACTTCTTGGCCTCA (SEQ ID NO: 65)	433
OtS2-C3	GCGCTGGGATTTTACGTGTG (SEQ ID NO: 66)	CCTTCCTGAAAACATGCCTAGG (SEQ ID NO: 67)	442
OtS2-B6	TTTAAAAGGGAGGGGTGGCA (SEQ ID NO: 68)	TGGTGAAGGGTCTCTAGGGCA (SEQ ID NO: 69)	347
OtS2-A12	GCATCTGTCGCTTGGAAGGA (SEQ ID NO: 70)	GCAAAACGTCTCCCTCCACC (SEQ ID NO: 71)	353
OtS2-B3	AATGGGACTTCATGGCCTCC (SEQ ID NO: 72)	GGCCGATTCCTTTGCAGAAA (SEQ ID NO: 73)	375
OtS2-A5	AGCCCTGGACTGCAAAGCTC (SEQ ID NO: 74)	GCCTGGGCTGGGTAACAAGA (SEQ ID NO: 75)	298
OtS2-C4	TGTTTACAGACTTTGCAACC (SEQ ID NO: 76)	CATCAAGTCTGGTCTCTGAG (SEQ ID NO: 77)	307
R1-OS-B1-E5	TTCTTTGTTACCTCAGGGGC (SEQ ID NO: 78)	TTGCTGGCTTCTGTGACATG (SEQ ID NO: 79)	250
OtS2-C5	GTGTTTAGCATCTGAGCCTG (SEQ ID NO: 80)	AGATAACACCCCTGTGTGAG (SEQ ID NO: 81)	237
SvO2-1-F1	AGTGGGGGACATGAGGGTTGGC	GGCTGGCTCTGGCTCTGCTTTT (SEQ ID	855

Signature sequence	**sense primer "forward"**	**anti-sense primer "reverse"**	**product length (base pairs)**
	(SEQ ID NO: 82)	NO: 83)	
R1-OS-B1-D6	GCAAGCTGATTTTCAGGCTGCC (SEQ ID NO: 84)	GGCTGCTGGGCATTTTGGAAAA (SEQ ID NO: 85)	383
R1-SO-R1-A12	ATTCAGTGCTTGCCGGAT (SEQ ID NO: 86)	TGGTTGGGTGCACGATGT (SEQ ID NO: 87)	233
R1-OS-B1-B1	GGTGATCAAAAATGCAGACATG (SEQ ID NO: 88)	GAACAGAAGAATGGCCACCT (SEQ ID NO: 89)	241
HPRT	GTTGGATACAGGCCAGACTTTGTT G (SEQ ID NO: 90) or AGTCCCAGCGTCGTGATTAGCGAT GA (SEQ ID NO: 92)	GATTCAACTTGCGCTCATCTTAGGC (SEQ ID NO: 91) or TGGCCTGTATCCAACACTTCGAGAGGT (SEQ ID NO: 93)	158 or 516

1: primers used for PCR reactions of cDNA obtained from cell lines (see Table 6).

2: primers used for cDNA obtained from mouse tissues (see Table 5) designed to detect APLP2 gene without or with the Kunitz protease inhibitor (KPI) domain.

[00197] Table 5: Difference in expression of the indicated gene in lung tissue, trachea, thoracic lymph nodes (TLN) and dorsal-root ganglia (DRG) of OVA-sensitized mice challenged with OVA versus saline. The value indicated in the table represents the difference in the number of two-fold dilution steps. A value of "3" means that the expression in OVA-challenged mice is at least 2^3 (=8) times higher than in saline-challenged mice. A value of "-3" means that the expression in OVA-challenged mice is at least eight times lower than in saline-challenged mice. See Example 1 for a detailed explanation.

Signature sequence	Trachea	Lung	DRG	TLN
OtS1-B7	0	0	13	0
SvO2-1-B7				
+KPI	0	-2	3	3
- KPI	0	-2	-2	3
SvO2-1-D8	0	0	0	2
OtS2-A6	ND	ND	0	-1
R1-OS-B1-D3	0	0	-1	0
R1-OS-B1-A1	2	0	2	0
R1-SO-R1-C11	12	12	ND	0
EST AA726662	4	0	5	-2
EST W41083	ND	ND	ND	ND
SvO2-1-D10	0	0	0	0
SvO2-1-A11	0	2	3	3
SvO2-1-C8	1	0	4	1
SvO2-1-E6	0	1	1	1
OtS2-D3	-1	3	0	0
OtS2-D10	-1	2	1	-2
R1-OS-B1-C3	0	-1	10	0
OtS2-G2	ND	ND	0	-1
R1-OS-B1-H6	0	1	-2	2
R1-OS-B1-A3	-1	1	-1	-3
R1-OS-B1-C4	0	0	2	0
R1-OS-B1-A5	0	2	-3	-3
R1-OS-B1-B2	0	0	-2	1
OtS2-C1	0	0	-2	2
OtS2-D7	0	2	0	2
OtS2-B9	ND	ND	3	0

Signature sequence	Trachea	Lung	DRG	TLN
OtS2-A9	1	3	-1	1
R1-OS-B1-C6	0	1	1	1
OtS2-C3	3	1	-1	-1
OtS2-B6	0	1	0	0
OtS2-A12	0	3	0	0
OtS2-B3	-1	-1	1	0
OtS2-A5	2	-1	-1	-1
OtS2-C4	0	-1	-1	-2
R1-OS-B1-E5	-1	0	-2	2
OtS2-C5	0	-2	1	0
SvO2-1-F1	1	-1	0	1
R1-OS-B1-D6				
383 bp	0	0	4	0
310 bp	absent	absent	-5	1

ND: Not determined

[00198] Table 6: Expression of the specified gene in the indicated murine cell line. "+" indicates that the gene is expressed in the cell line; "-" indicates absence of expression of the specified gene in the cell line.

Signature sequence	P815	CFTL12	EL4	3D054.8	DO11.10	A20	J774A.1	RAW 264.7	C10	3T3	DC
OtS1-B7	-	+	-	-	-	-	-	-	-	-	+
SvO2-1-B7	+	+	+	+	+	+	+	+	+	+	+
SvO2-1-D8	+	+	+	+	+	+	+	+	+	+	+
OtS2-A6	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-D3	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-A1	-	-	-	-	-	-	-	-	-	-	-
R1-SO-R1-C11	-	-	-	-	-	+	+[3]	+[3]	-	-	-
EST AA726662	-	-	-	-	-	+	+[3]	-	-	+	-
EST W41083	-	+	-	-	-	-	+	+	-	+[1]	-
mCaCC	ND	ND	ND	-	-	-	-	-	+	-	ND
SvO2-1-D10	+[1]	-	-	-	-	+	+	+	-	-	-
SvO2-1-A11	+	+	+	+	+	+	+	+	+	+	+
SvO2-1-C8	+	+	+	+	+	+	+	+	+	+	+
SvO2-1-E6	+	+	+	+	+	+	+	+	+	+	+
OtS2-D3	+	+	+	+	+	+	+	+	-	+	-
OtS2-D10	-	ND	-	-	+	+	-	+	+	+	ND
mCD59	+	+	-	+	+	+	-	+	+	+	+
5'UTR variant	-	-	-	-	-	+	-	+	-	-	-
R1-OS-B1-C3	+	+	+	+	+	+	+	+	+	-	+[1]

Signature sequence	P815	CFTL12	EL4	3D054.8	DO11.10	A20	J774A.1	RAW 264.7	C10	3T3	DC
OtS2-G2	+[1]	+	+[1]	-	-	-	-	-	-	-	+
R1-OS-B1-H6	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-A3	+	-	-	-	-	+[2]	-	-	-	-	-
R1-OS-B1-C4	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-A5	+	-	+	-	-	-	-	+	-	-	-
R1-OS-B1-B2	+	+	+	+	+	+	+	+	+	+	+
OtS2-C1	+	+	+	+	+	+	+	+	+	+	+
OtS2-D7	+	+	+	+	+	+	+	+	+	+	+
OtS2-B9	-	-	+	-	-	-	+	-	-	-	+
OtS2-A9	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-C6	+	+	+	+	+	+	+	+	+	+	+
OtS2-C3	+	+	+	+	+	+	+	+	+	+	+
OtS2-B6	+	+	+	+	+	+	+	+	+	+	+
OtS2-A12	+	+	+	+	+	+	+	+	+	-	+
OtS2-B3	+	+	+	+	+	+	+	+	+	+	+
OtS2-A5	+[1]	+	+	+	+	+	+	+	+	+	-
OtS2-C4	+	+	+	+	+	+	+	+	+	+	+
R1-OS-B1-E5	+	+	-	+	+	+	+[3]	+	+	+	+
OtS2-C5	+	+	+	+	+	+	+	+	+	-	-
SvO2-1-F1	+	+	+	+	+	+	+	+	+	+	+

Signature sequence	P815	CFTL12	EL4	3D054.8	DO11.10	A20	J774A.1	RAW 264.7	C10	3T3	DC
R1-OS-B1-D6	-	-	-	-	-	-	-	-	-	-	-
R1-SO-R1-A12	ND	ND	ND	+	+	-	-	-	+	-	ND
R1-OS-B1-B1	ND	ND	ND	+	+	+	+	+	+	+	ND

ND: Not determined.

1: only after *in vitro* activation with PMA (10 ng/ml) for three hours.

2: only after *in vitro* activation with an activating anti-CD40 monoclonal antibody (clone 3/23; 1 μg/ml) for three hours.

3: only after *in vitro* activation with lipopolysaccharide (1 μg/ml) and interferon-γ (50 U/ml) for three hours.

Murine cell lines used: P815: Mast cell

CFTL12: Mast cell

EL4: T-lymphocyte

3DO54.8: T-helper lymphocyte

DO11.10: T-helper lymphocyte

A20: B-lymphocyte

J774A.1: Macrophage, Monocyte

RAW264.7: Macrophage, Monocyte

C10: Lung type II epithelial cell

3T3: Fibroblast

DC: Primary bone marrow-derived dendritic cell

[00199] Table 7: Annotation of Contig1A (19619 bp) of the gene comprising the OtS1-B7 fragment. Numbers refer to the nucleotide position in Contig 1A.

3633	3654	5'-UTR	
3655	3657	ATG-startcodon	
3655	3700	46 bp exon 1	
3701	3808		108 bp intron 1
3809	3895	87 bp exon 2	
3896	4420		525 bp intron 2
4421	4510	90 bp exon 3	
4511	5011		501 bp intron 3
5012	5092	81 bp exon 4	
5093	6149		1057 bp intron 4
6150	6236	87 bp exon 5	
6237	7064		828 bp intron 5
7065	7151	87 bp exon 6	
7152	8376		1225 bp intron 6
8377	8463	87 bp exon 7	
8464	8954		491 bp intron 7
8955	9106	152 bp exon 8	
9107	10385		1279 bp intron 8
10386	10495	110 bp exon 9	
10496	11617		1122 bp intron 9
11618	11966	349 bp exon 10	
11618	12784	1167 bp exon 10A	
11766	11768	TAG-stopcodon	
11769	11966	3'-UTR	
11946	11950	poly adenylationsite	
11769	12784	3'-UTR	
12769	12773	poly adenylationsite	
		differential splicing yields two mRNAs of ca 1198 and 2016 bp, respectively, both encoding the same 325 bp ORF	

[00200] Table 8: Primers used for the characterization of the gene comprising OtS1-B7. "F" in the primer name refers to forward or sense primer; "R" in the primer name refers to reverse or anti-sense primer.

Position in contig1A	**primer name**	**sequence**
3642-3659	0055-FOTS1-B7F	GACAGCGGCAACCATGAG (SEQ ID NO: 94)
3647-3673	0054-FOTS1-B7F	CGGCAACCATGAGTGACTCCACAGAAG (SEQ ID NO: 95)
3655-3684	0047-OTS1-B7F	AAGAACAGGAAGGAGAGCAGCTGCAGGAC (SEQ ID NO: 96)
4450-4478	0048-OtS1-B7-R	AGCTGGGTCAGTTCCTGGAGGATCTTCTCT (SEQ ID NO: 97)
5049-5078	0049-OtS1-B7-R	AGCTGGGTCAGTTCCTGGAGGATCTTCTCT (SEQ ID NO: 98)
5078-5092 + 6150-6163	0050-OtS1-B7-R	GGGGATCCTGGACGTAAGCTCATCTGTCA (SEQ ID NO: 99)
4506-4510 + 5012-5038	0017-F-OtS1-B7	CCAAGTCTCCAAAACCCCAAATACCGAGAGGC (SEQ ID NO: 100)
5012-5041	0032-OTS1-B7F	TCTCCAAAACCCCAAATACCGAGAGGCAGA (SEQ ID NO: 101)
10393-10422	0033-OTS1-B7F	TGCAGCAGGCTTCTAAGGCTAAAGGACCAA (SEQ ID NO: 102)
11636-11665	0034-OTS1-B7R	TCCTCACCGATGTTGTTAGGCTCCCCTCTA (SEQ ID NO: 103)
11666-11695	0010-R-OtS1-B7	CAGCCATCCCCAGCAAATTCGACACAGTCT (SEQ ID NO: 104)
11682-11711	0052-OtS1-B7-F	GCTGGGGATGGCTGGAATGACTCTAAATGT (SEQ ID NO: 105)
11735-11763	0053-OtS1-B7-F	CAAGAAGTCTGCAACCCCATGCACTGAAG (SEQ ID NO:

		106)
11769-11796	0056-OtS1-B7-R	ATGGCATGAAGGTAGGAGCGGAGATGAG (SEQ ID NO: 107)
11781-11828	0057-OtS1-B7-R	CGAAAGTGAGGCACATCCAT (SEQ ID NO: 108)
11861-11891	0051-OtS1-B7-R	AAGAAGAATCCCAGAGCCTTTTTCACGATCC (SEQ ID NO: 109)
11862-11881	0124-OtS1-B7-F	GATCGTGAAAAAGGCTCTGG (SEQ ID NO: 110)
12183-12202	0126-OtS1-B7-F	TGGCTAGATGTTCCCACCTC (SEQ ID NO: 111)
12832-12851	0125-OtS1-B7-R	TTCCTGCAGGGATGAGCTAC (SEQ ID NO: 112)
12558-12577	0127-OtS1-B7-R	TCAGATCACCAGCCTTGCTA (SEQ ID NO: 113)
12753-12779	0140-OtS1-B7-R	CAGGACTTTATTACAGCAACAGTAAAC (SEQ ID NO: 114)

[00201] Table 10: Primer pairs used for PCR analysis of human calcium-activated chloride channel family members and HPRT in human H292 lung epithelial cells.

Gene	**sense primer**	**anti-sense primer**	**product length (base pairs)**
CLCA1	TGCAGACAGTTGAGCTGGGGTCCT (SEQ ID NO: 115)	CCCCAAAAGCATCAATGAGGCC (SEQ ID NO: 116)	417
CLCA2	AAATTCATACCTTCGTGGGCATTGC (SEQ ID NO: 117)	CTGGCCTGCCACGTAACTAGAAACA (SEQ ID NO: 118)	568
CLCA4	GCAAAACATTTCCTGCTGCAGACTG (SEQ ID NO: 119)	TGAGGCCATTGTTCTGAGCCTTCATC (SEQ ID NO: 120)	421
HPRT	TGCTGAGGATTTGGAAAGGGTGTTT (SEQ	TGACCAAGGAAAGCAAAGTCTGCAT	368

	ID NO: 121)	(SEQ ID NO: 122)	

[00202] **Table 11:** members of the calcium-activated chloride channel family.

Human gene/protein	Murine homolog
CLCA1 / CaCC1	Gob-5
CLCA2 / CaCC3 / CaCL2	EST AA726662
CLCA3	CaCC / CLCA1
CLCA4 / CaCC2	EST W41083

[00203] **Table 9A:** Serum levels of OVA-specific IgE (Units/ml). Balb/c mice (n=5 or 6 per group) were treated with control antibody or ERTR9 antibody prior to sensitization and prior to the first of three OVA inhalation challenges (group I). Levels of OVA-specific IgE were measured before and after OVA inhalation challenge. Values are expressed as mean ± SEM. Serum IgE levels after challenge are significantly increased *$P<0.05$ as determined by the student's t-test.

Treatment	Control	ERTR9
Before challenge	606.4 ± 59.2	484.2 ± 62.1
After challenge	1739.6 ± 202.3*	992.3 ± 301.7

[00204] **Table 9B:** Serum levels of OVA-specific IgE (Units/ml). Balb/c mice (n=5 or 6 per group) were treated with control antibody or ERTR9 antibody prior to the first of three OVA inhalation challenges (group II). Levels of OVA-specific IgE were measured before and after OVA inhalation challenge. Values are expressed as mean ± SEM. Serum IgE levels after challenge are significantly increased *$P<0.05$ as determined by the student's t-test.

Treatment	Control	ERTR9
Before challenge	203.6 ± 40.62	190.0 ± 23.8
After challenge	17257.2 ± 4088.7*	9684.7 ± 2596.0*